Antimicrobial Susceptibility Testing with EUCAST.

Gunnar Kahlmeter
EUCAST Past chair
Currently Technical Data Coordinator and Webmaster
Sweden

Why EUCAST

1. Structure
   – organised by profession in liaison with regulatory authorities.
2. No commercial influence or dependancy
3. Effective decision process
   - 5 meetings per year
   - Rapid turnaround time on questions
   - EUCAST does not wait for FDA, Companies or Manufacturers
   - National influence via NAC and rep on General and Steering Committees
4. Public consultation process
5. Formal revision process
6. All data used for decisions are made publicly available (Rationale Documents, Calibration etc)
7. Dynamic breakpoint table with multiple functions
8. Lab.facilities for training, development and revision
9. Open free of charge website (www.eucast.org)
10. Increasing international reach

EUCAST Milestones

- 2002 when the national committees decided to take joint responsibility for a European standard.
- 2004 when EMA agreed to recognize EUCAST as its breakpoint committee.
- 2008 when existing antimicrobials had EUCAST breakpoints
- 2008 with the decision to develop a EUCAST disk diffusion test
- 2014 when the CA-SFM abandoned the french disk diffusion test
- 2014 when many countries outside Europe decided to turn to EUCAST and leave CLSI.
- 2016 when the BSAC abandoned the UK disk diffusion test.
- 2016 with the publication of the uneven quality of disks from 9 manufacturers.

EUCAST
EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING
European Society of Clinical Microbiology and Infectious Diseases

www.eucast.org

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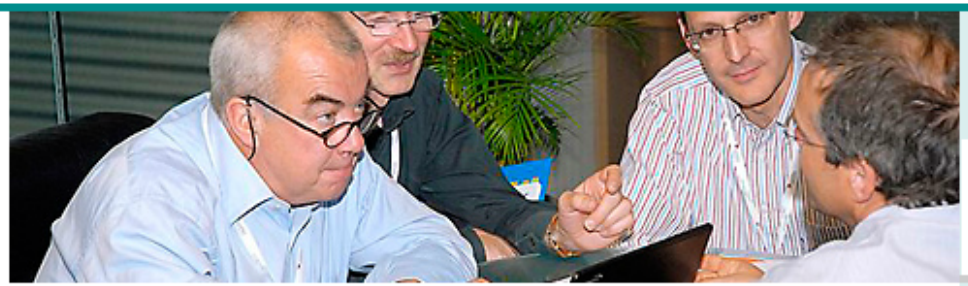
Website changes



search term Search

QUICK NAVIGATION

The European Committee on Antimicrobial Susceptibility Testing - EUCAST

EUCAST is a standing committee jointly organized by ESCMID, ECDC and European national breakpoint committees. EUCAST deals with breakpoints and technical aspects of phenotypic in vitro antimicrobial susceptibility testing and functions as the breakpoint committee of EMA and ECDC. EUCAST does not deal with antibiotic policies, surveillance or containment of resistance or infection control. The Steering Committee is the decision making body. It is supported by a General Committee with representatives from European and other countries, FESCI and ISC. The Steering Committee also consults on EUCAST proposals with experts within the fields of infectious diseases and microbiology, pharmaceutical companies and susceptibility testing device manufacturers.

EUCAST has a subcommittee on antifungal susceptibility testing and on methods for detection of resistance mechanisms of clinical and/or epidemiological importance.

Subcommittees on expert rules for antimicrobial susceptibility testing and antimicrobial susceptibility testing of anaerobes have completed their tasks and have been disbanded.

Most antimicrobial MIC breakpoints in Europe have been harmonised by EUCAST. Breakpoints for new agents are set as part of the licensing process for new agents through EMA. EUCAST breakpoints are available in devices for automated susceptibility testing but with some limitations, depending on the system. A disk diffusion susceptibility test method calibrated to EUCAST MIC breakpoints is also available.

EUCAST News

13 Mar 2014
ESCMID Post Graduate Course

09 Mar 2014
EUCAST Steering Committee positions for 2014 - 2016

26 Feb 2014
FAQ - updated 2014-02-28

27 Jan 2014
Danish NAC presented

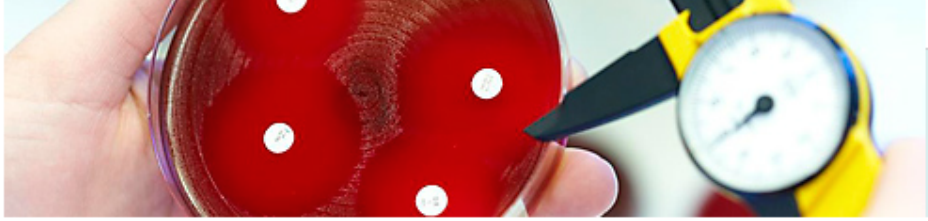
22 Jan 2014
SOP 7.0 Preparation and handling of EUCAST minutes (publ 2014-01-22)

About Newsfeeds

ESCMID EUROPEAN SOCIETY OF CLINICAL MICROBIOLOGY

Economy now and future!

- All EUCAST output is free of charge.
- ECDC finances the committee work on a contractual basis
- ESCMID finances all technical development (methods, MIC distributions etc)
- Industry does not contribute financially and can only influence decisions through the consultation process.



Development and validation of EUCAST Disk Diffusion breakpoints

The EUCAST Disk Diffusion test was developed by EUCAST during 2009 - 2013 under the auspices of ESCMID and with the help of many laboratories. The help of these laboratories is gratefully acknowledged.

The files below list material and graphs used for determining zone diameter breakpoints to match MIC breakpoints (Example 1). Also all MIC and zone diameter data are entered in the EUCAST MIC- and Zone diameter distribution program (Example 2).

All files were updated 2016-01-28 (breakpoints checked against Breakpoint table 6.0 and new graphs added). Older versions can be obtained from the web master.

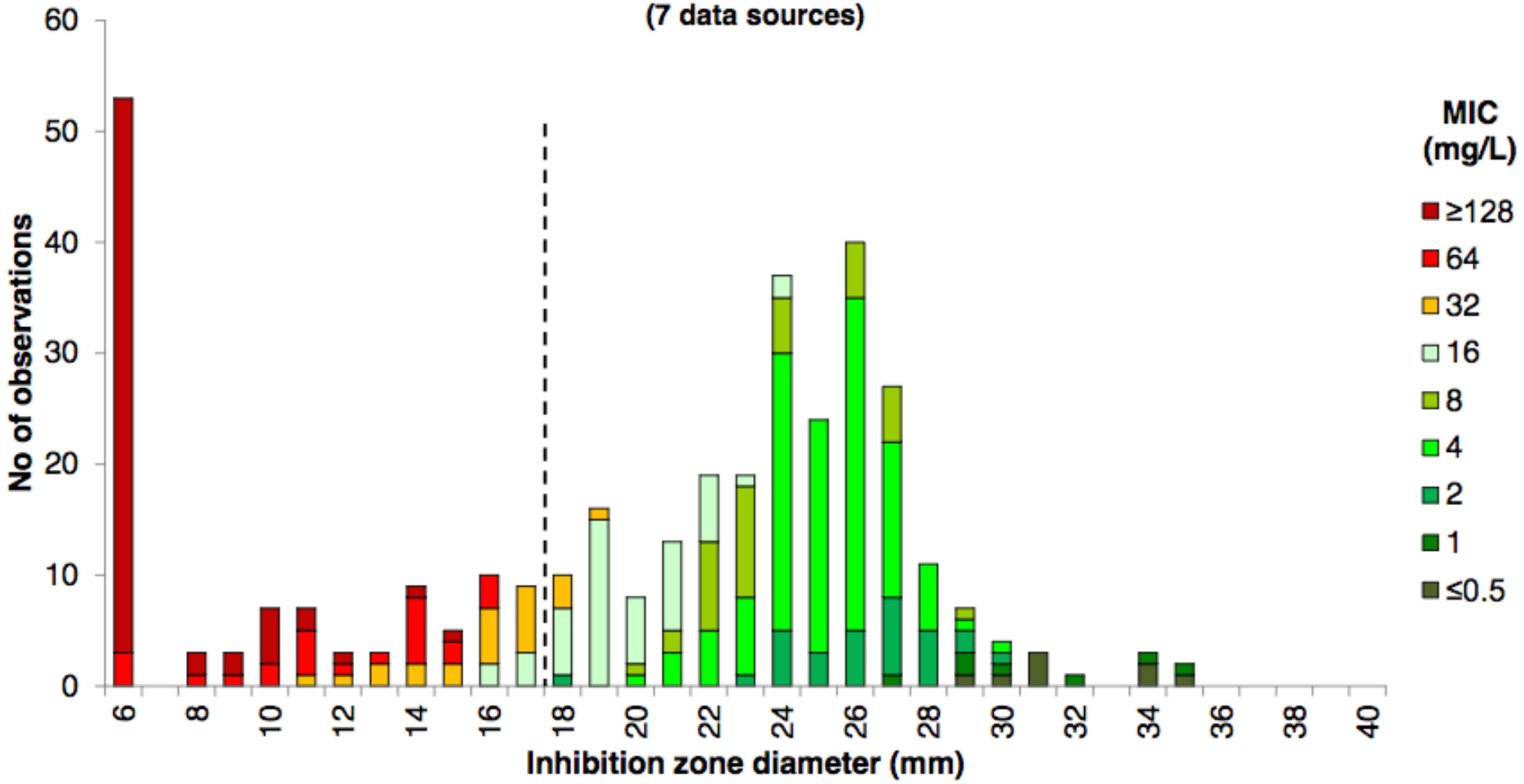
1. Enterobacteriaceae (file from 2015; new file under preparation)
2. Salmonella spp
3. Pseudomonas aeruginosa
4. Pseudomonas non-aeruginosa
5. Staphylococcus aureus
6. Staphylococcus, coagulase-negative
7. Staphylococcus saprophyticus
8. Staphylococcus pseudintermedius
9. Enterococcus spp.
10. Streptococcus pyogenes (Group A)
11. Streptococcus agalactiae
12. Streptococcus pneumoniae
13. Streptococcus pneumoniae - screen for beta-lactam resistance
14. Viridans Group Streptococci
15. Haemophilus influenzae (file from 2015, new file under preparation)
16. Haemophilus influenzae - screen for betalactam resistance (file from 2015, new file under preparation)
17. Moraxella catarrhalis
18. Listeria monocytogenes
19. Pasteurella multocida
20. Campylobacter jejuni and C. coli
21. Corynebacterium spp.

Calibration files

- MIC (broth microdilution, ISO) and zone diameter data (EUCAST) used by EUCAST to validate disk diffusion breakpoints.
- The organisms used for this is a mixture of fresh clinical isolates and collections of organisms with defined and diverse resistance mechanisms.
- Organisms with resistance mechanisms which place them in the area between susceptible and resistant are overrepresented.
- This makes these graphs show the worst-case-scenario!

Piperacillin-tazobactam 30-6 μg vs. MIC
Pseudomonas aeruginosa, 249 isolates (356 correlates)

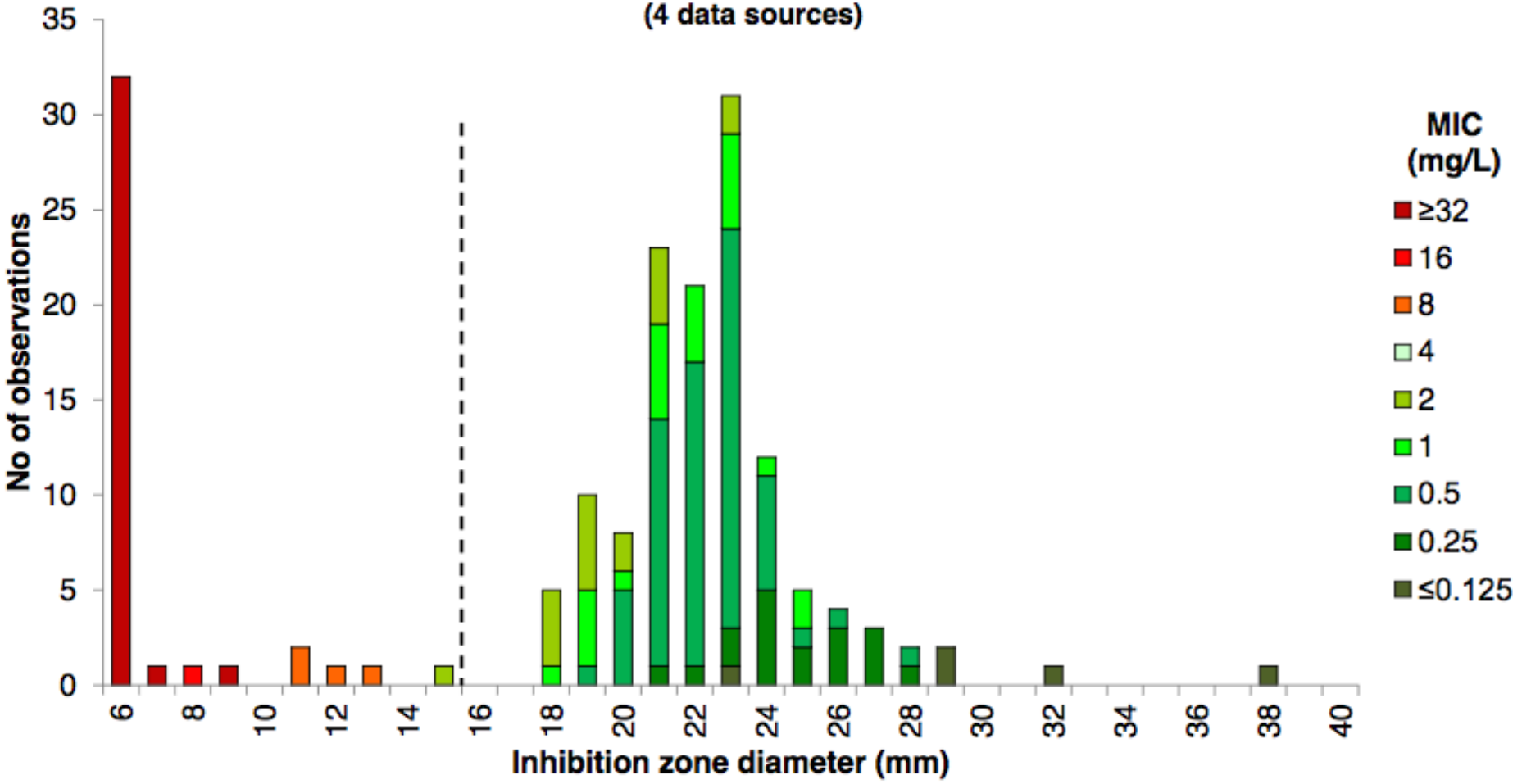
(7 data sources)



Breakpoints		ECOFF
MIC	S≤16, R>16 mg/L	16 mg/L
Zone diameter	S≥18, R<18 mm	

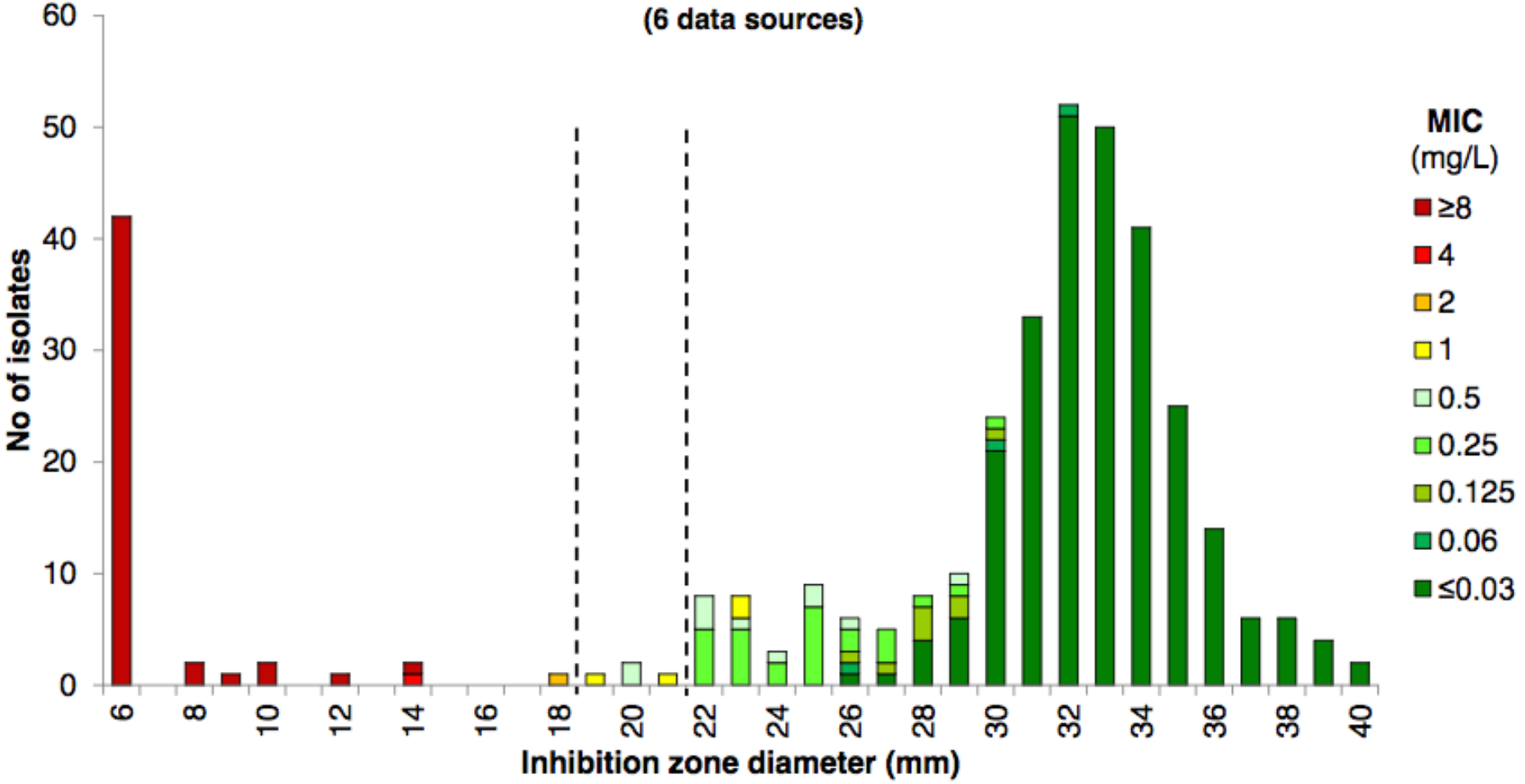
Tobramycin 10 μg vs. MIC
Pseudomonas aeruginosa, 168 isolates

(4 data sources)



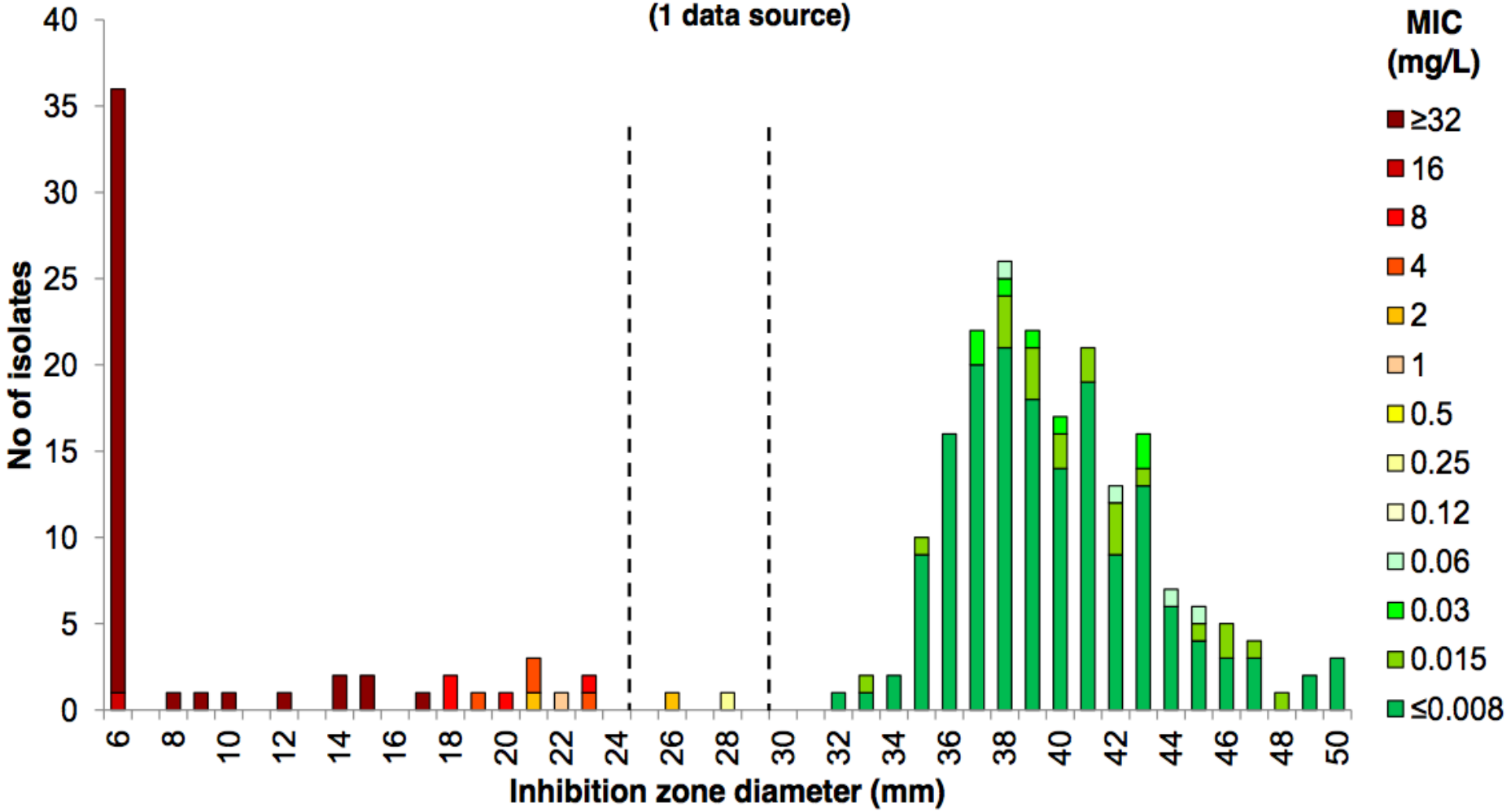
Breakpoints		ECOFF
MIC	S≤4, R>4 mg/L	2 mg/L
Zone diameter	S≥16, R<16 mm	

Ciprofloxacin 5 µg vs. MIC
E. coli, 369 clinical isolates



Breakpoints		ECOFF
MIC	S≤0.5, R>1 mg/L	0.06 mg/L
Zone diameter	S≥22, R<19 mm	

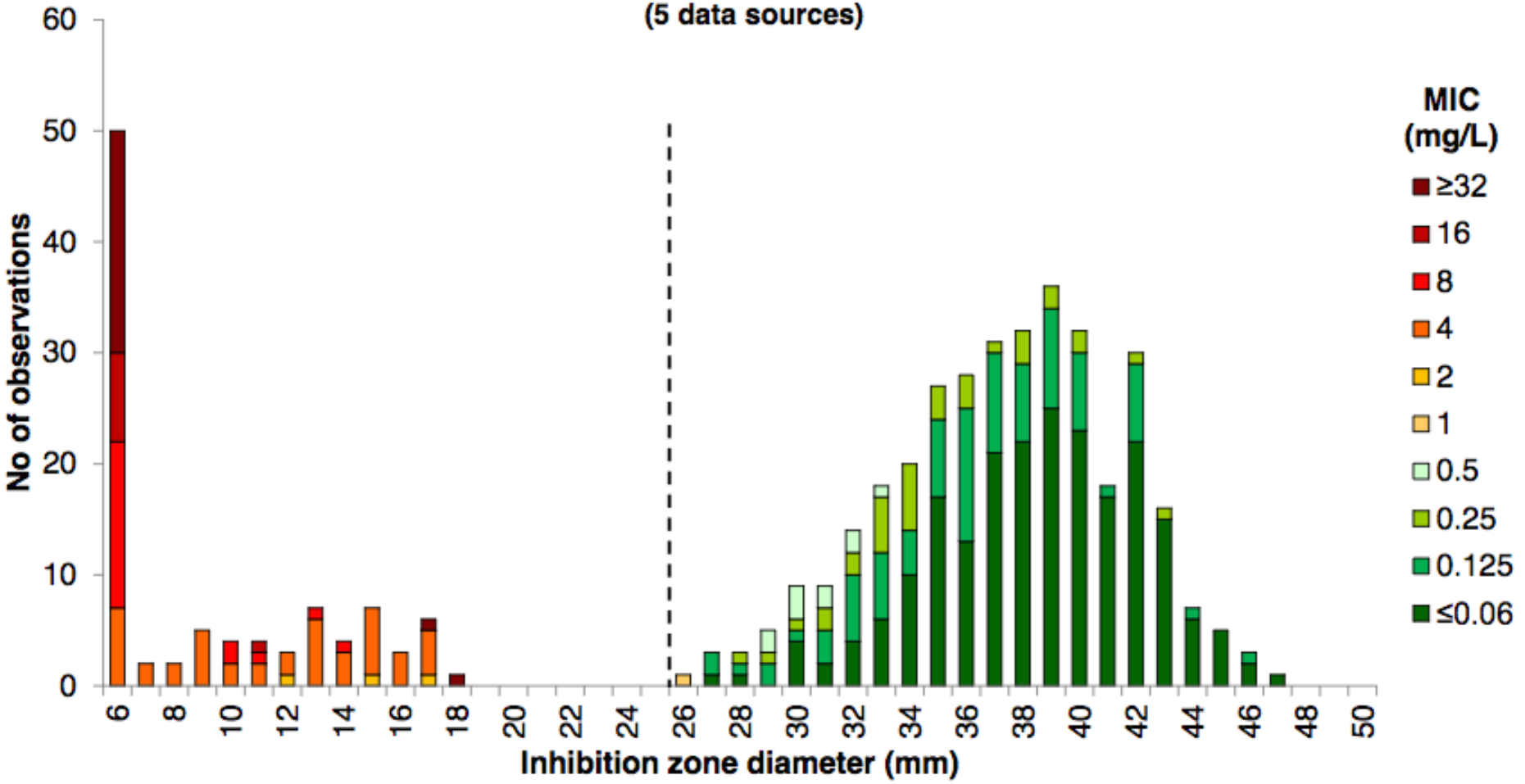
Rifampicin 5 *µg* vs. MIC
Corynebacterium spp., 253 clinical isolates



Breakpoints	
MIC	S≤0.06, R>0.5 mg/L
Zone diameter	S≥30, R<25 mm

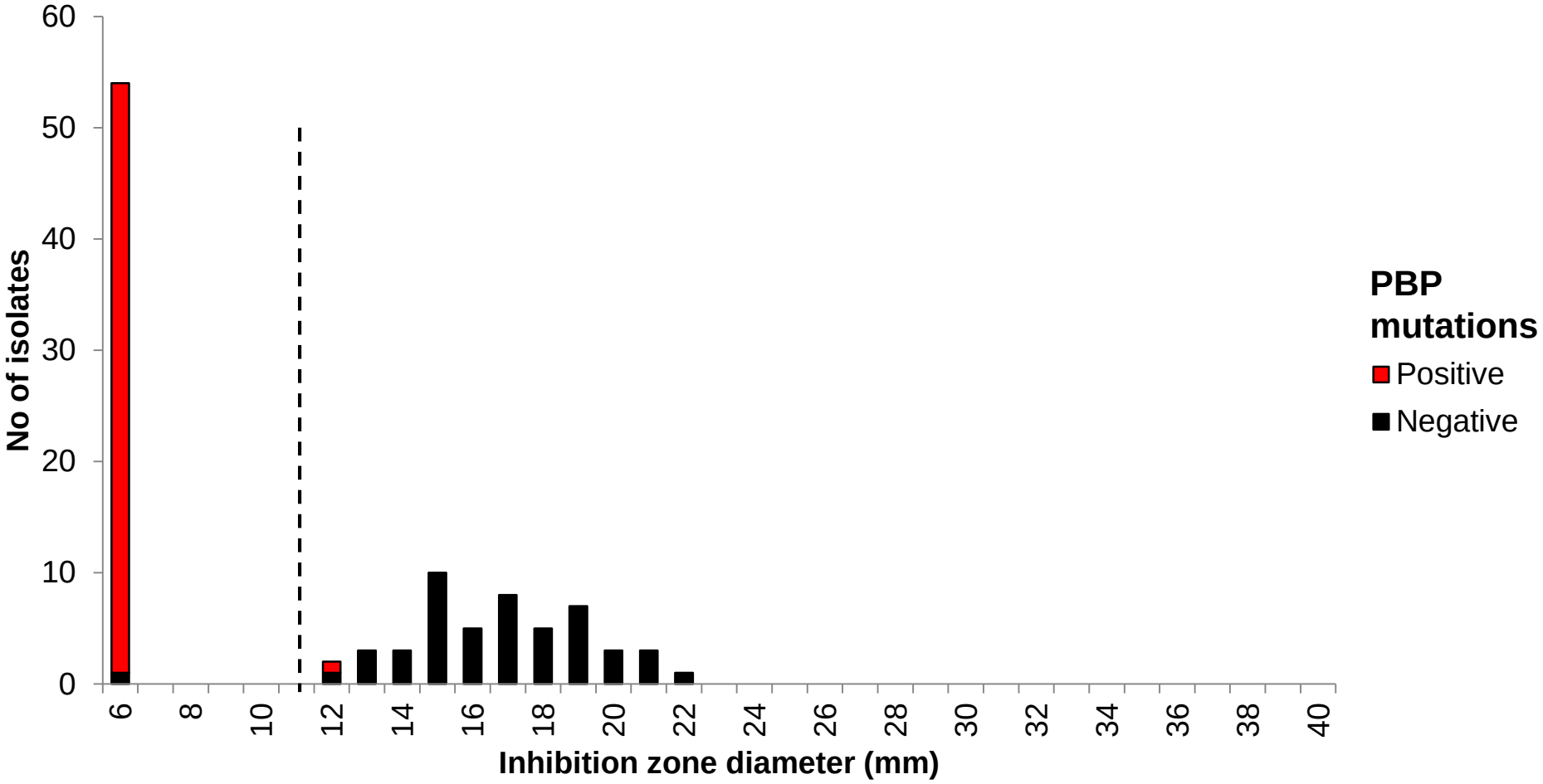
Ciprofloxacin 5 μg vs. MIC
C. jejuni and *coli*, 360 isolates (446 correlates)

(5 data sources)



Breakpoints		ECOFF
MIC	S≤0.5, R>0.5 mg/L	0.5 mg/L
Zone diameter	S≥26, R<26 mm	

Benzylpenicillin 1 unit vs. PBP mutations
H. influenzae, 104 β-lactamase negative clinical isolates

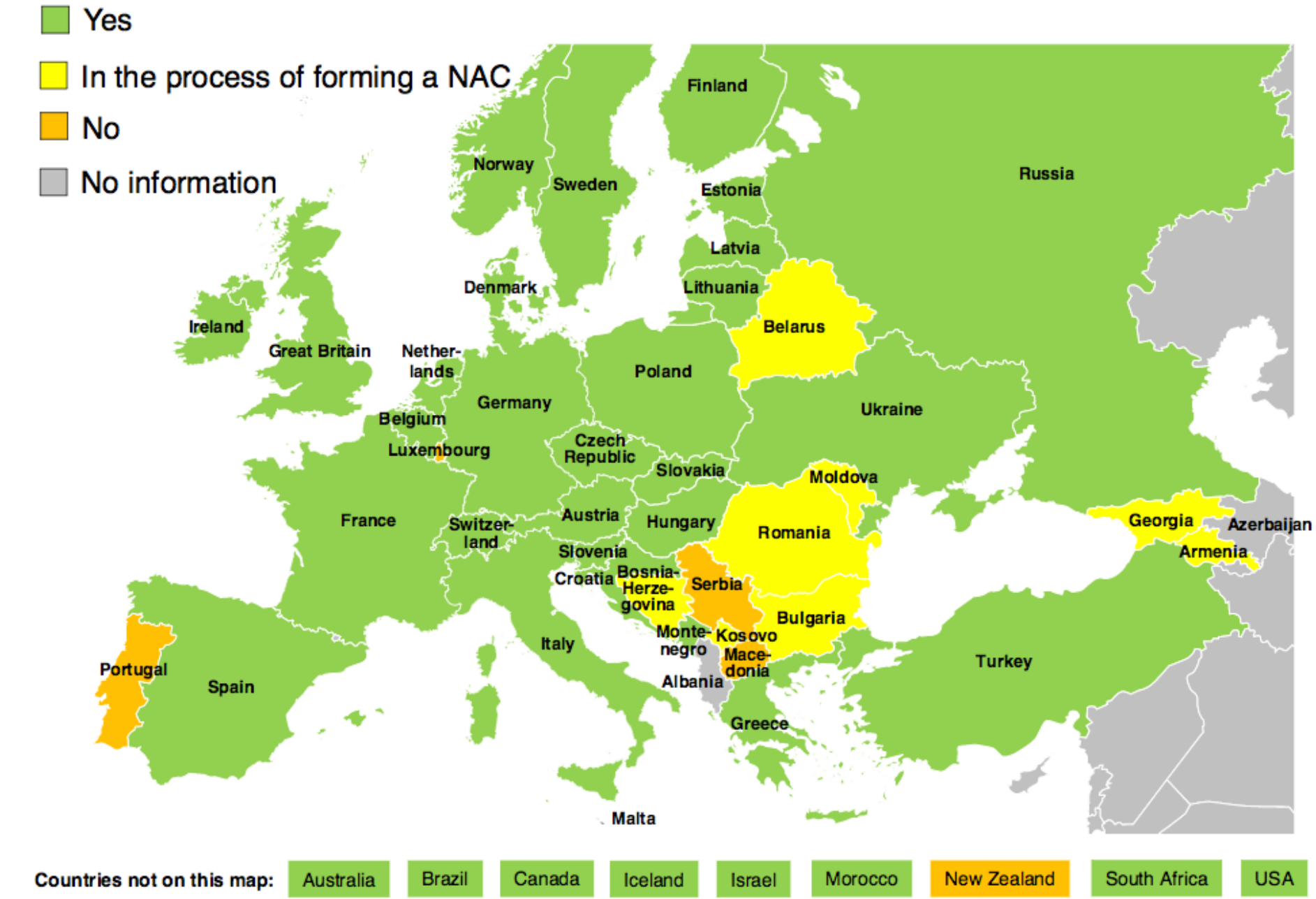


Benzylpenicillin zone diameter screen breakpoint (S≥12, R<12 mm) to detect all betalactam resistance.

EUCAST today?

- We aimed to solve a hopeless European situation where countries inside EU were using 7 different standards.
- We did not aim to convince anyone outside Europe to go EUCAST.
- We would have been happy to join forces with CLSI in the beginning but CLSI was not interested.
- And here we are….

National AST Committees (NACs), April 2016



National Antimicrobial Committees (NACs) outside Europe

Countries with a NAC operating under EUCAST standards

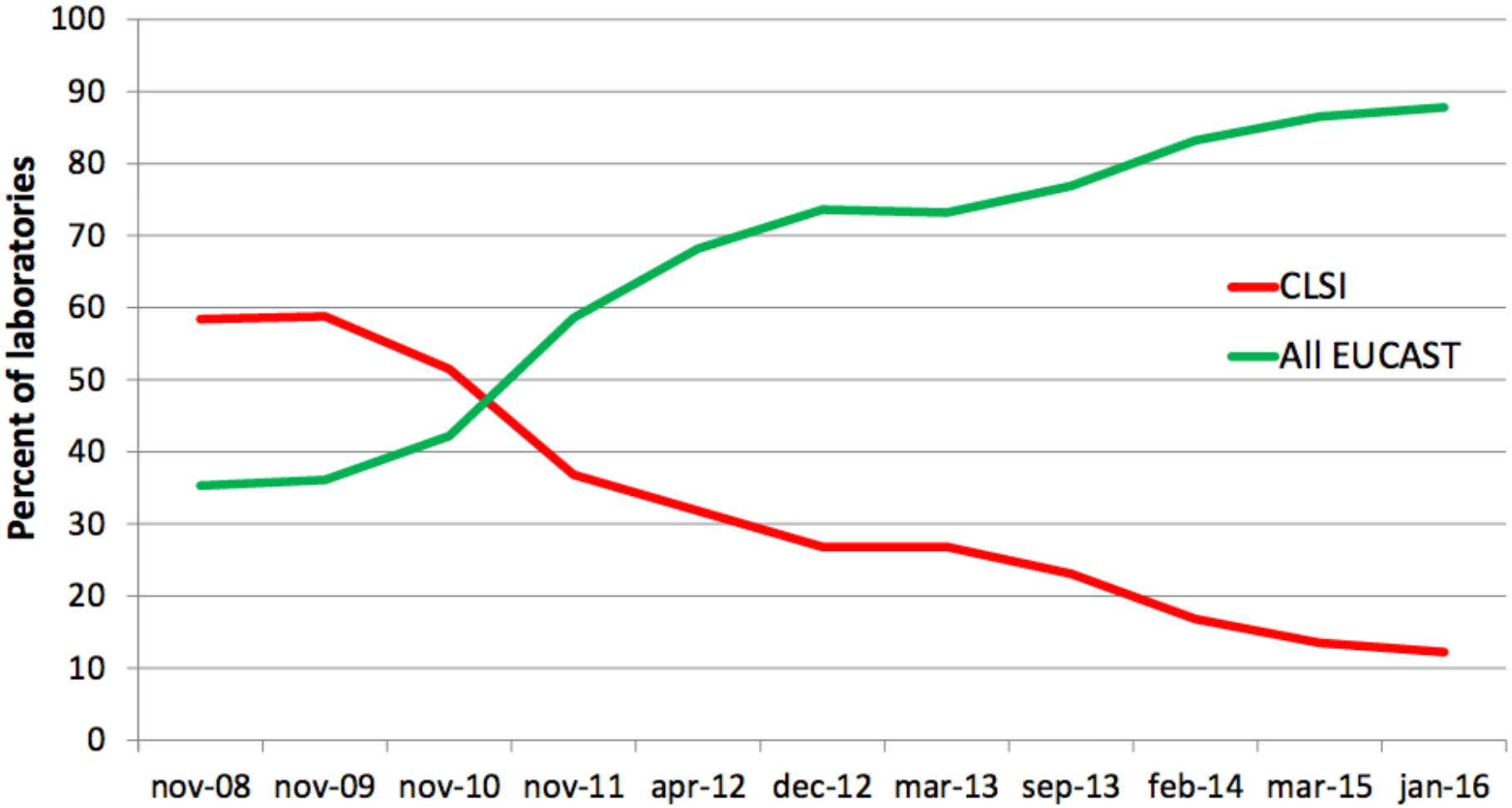
Countries with interest to establish a NAC under EUCAST standards

Implementation of EUCAST breakpoints, April 2016

Countries not on this map: Australia | Brazil | Canada | Iceland | Israel | Morocco | New Zealand | South Africa | USA

AST guidelines used in UK NEQAS External Quality Assurance

(630-750 participants per year from a total of 40 countries)

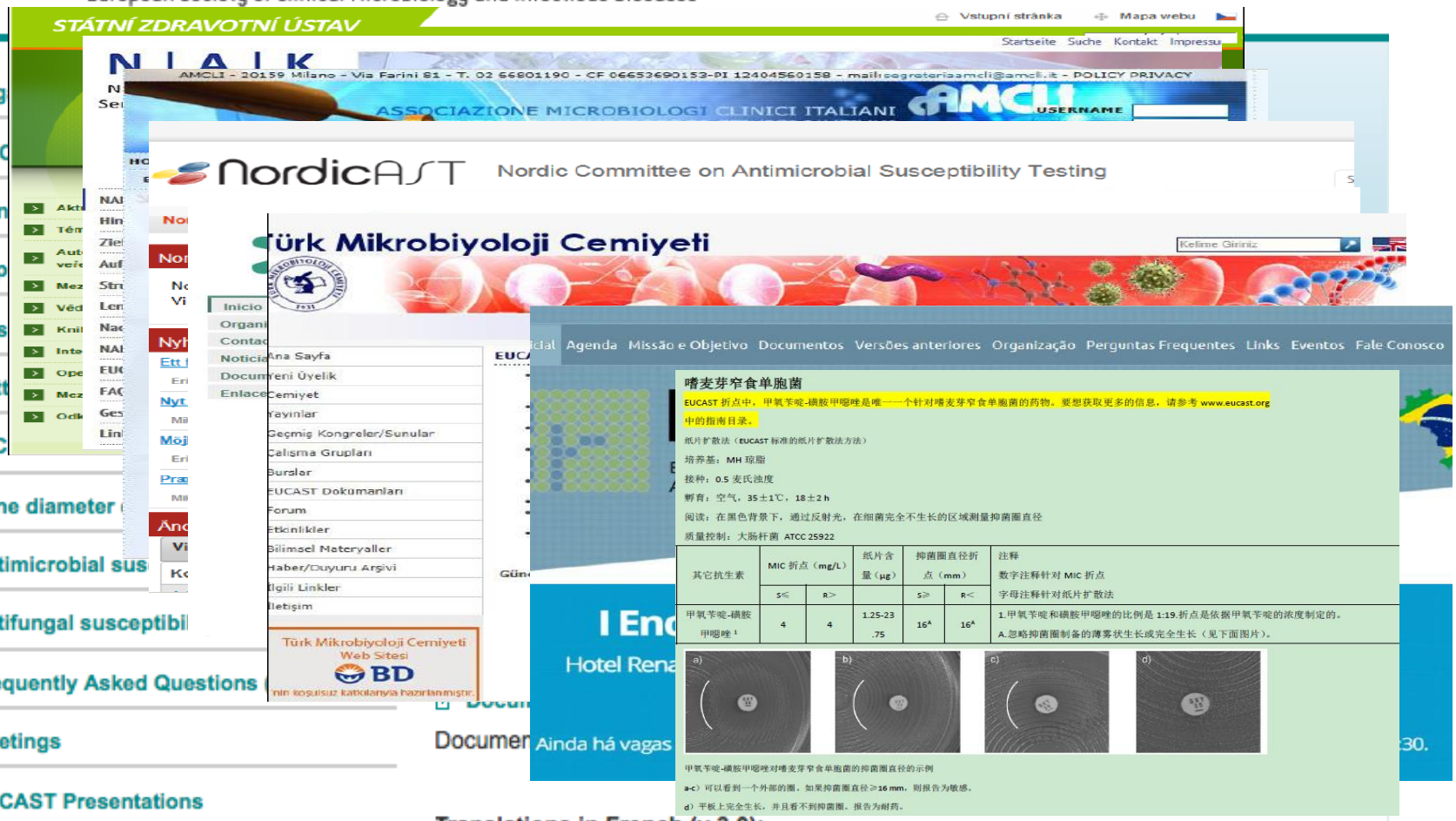


UK NEQAS

EUCAST

EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING

European Society of Clinical Microbiology and Infectious Diseases



- Org
- EUC
- Clin
- Exp
- Res
- Sett
- MIC
- Zone diameter
- Antimicrobial sus
- Antifungal susceptibi
- Frequently Asked Questions
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- EUCAST Presentations
- Documents
- **Translations**
- Information for industry
- Links
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Translations in French (v 3.0):

- Implementation guideline
- Media preparation

- EUCAST Disk Diffusion - Manual
- EUCAST Disk Diffusion - Slide Show
- EUCAST Disk Diffusion - Reading Guide

Dynamic breakpoint table with multiple functions

- The breakpoint table is downloadable from the website (Excel or PDF)
- Each agent is linked to its rationale document describing data behind breakpoint decisions.
- Each MIC breakpoint is linked to the relevant MIC distributions
- Each zone diameter breakpoint is linked to the relevant zone diameter distributions
- Doses of agents pertinent to the breakpoint(s) are listed
- PK/PD is available in a specific tab.

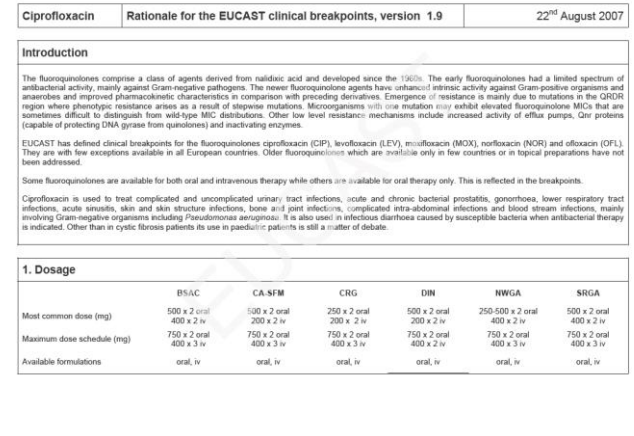
Links in EUCAST breakpoint table

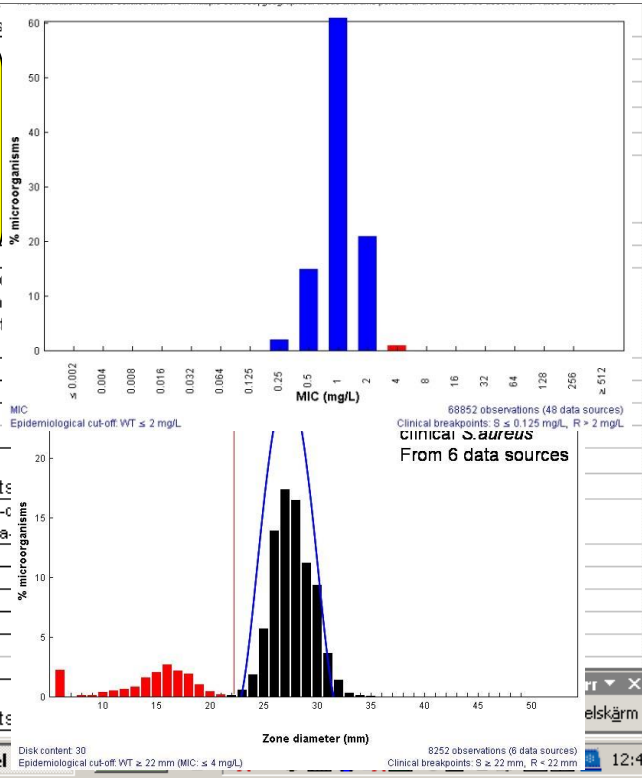
Click on antibiotic for **Rationale Document**

Click on MIC breakpoint for **MIC distributions**

Click on zone breakpoint for **zone diameter distributions**

	A	B	C	D	E	F
44						
45	**Carbapenems**					
46						
47						
48	Doripenem					
49	Ertapenem					
50	Imipenem[1]					
51	Meropenem	2	8	**10**	22	16
52						
53						
54	**Monobactams**	**MIC breakpoint**		**Disk**	**Zone diameter**		**Notes**
55		**S ≤**	**R>**	**content**	**S ≥**	**R<**	Numbers for comments on MIC bre
56							
57	Aztreonam[1]	1	8	**30**	25	21	**1**. The aztreonam breakpoints for Enterob clinically important beta-lactamases in Ente susceptible or intermediate with these breakpoints may want to use a test which specifically screens
58							
59							
60	**Fluoroquinolones**	**MIC breakpoint**		**Dis**			
61		**S ≤**	**R>**	**cont**			
62							
63	Ciprofloxacin[1]	0.5	1	5			
64	Levofloxacin	1	2	5			
65	Moxifloxacin	0.5	1	5			
66	**Halidixic acid (screen)**	Note[2]	Note[2]	**30**	16*	16*	**2/A**. Nalidixic acid may be used to screen for fluor breakpoint correlates to an MIC value of 16 mg/L in resistant to all fluoroquinolones. If other Enterobact
67	Norfloxacin	0.5	1	**10**	22	19	
68	Ofloxacin	0.5	1	5		19	
69							
70							
71	**Aminoglycosides**[1]	**MIC bre**					
72		**S ≤**					
73							
74	Amikacin	8					
75	Gentamicin	2					
76	Netilmicin	2					
77	Tobramycin	2					
78							
79							
80	**Glycopeptides**	**MIC breakpoint**		**Disk**	**Zone diameter**		**Notes**
81		**S ≤**	**R>**	**content**	**S ≥**	**R<**	Numbers for comments on MIC breakpoints





EUCAST Frequently Asked Questions

- Several questions per day via telephone or e-mail
- Answers to difficult questions are often prepared with input from several EUCAST colleagues
- Each question is given a personal e-mail reply
- Common “Questions and Answers” are anonymised and added to the EUCAST website “FAQ”, which is updated regularly

www.eucast.org

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EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING

European Society of Clinical Microbiology and Infectious Diseases

search term

Frequently Asked Questions (FAQ)

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- Organization
- EUCAST News
- Clinical breakpoints
- Expert rules and intrinsic resistance
- Resistance mechanisms
- Guidance documents
- MIC distributions and ECOFFs
- Zone distributions and ECOFFs
- AST of bacteria
- AST of mycobacteria
- AST of fungi
- AST of veterinary pathogens
- **Frequently Asked Questions (FAQ)**
- Meetings
- Presentations and statistics
- Documents

Frequently Asked Questions (FAQ)

Updated March 2016

Frequently Asked Questions - valid from 2015-03-23

The EUCAST secretariat receives many questions on subjects ranging from how we determine breakpoints, the MIC-distribution website, and the new disk diffusion methodology. We try to answer each question individually but also publish frequently asked questions and answers in a classical FAQ document. The file is updated at regular intervals.

Previous version of FAQ:

Frequently Asked Questions - valid 2014-02-26 - 2015-03-23

Frequently Asked Questions - valid 2013-04-24 - 2014-02-26

EUCAST vs. CLSI
….will it make a difference?

30 - 40 % agreement between EUCAST and CLSI breakpoints.

(Summary of agreement between **CLSI** and **EUCAST** breakpoint criteria for 2013)

Organisms	No. assessed		Same breakpoints		Overall agreement (%)
	Com-pounds	Criteria	Susceptible	Resistant	
Enterobacteriaceae	30	60	10	4	23.3%
P. aeruginosa	17	34	9	3	35.3%
Acinetobacter spp.	10	20	5	3	40.0%
Staphylococci	25	50	11	5	32.0%
Enterococci	5	10	2	2	40.0%
S. pneumoniae	27	60	11	11	36.7%
All results	**-**	**234**	**48**	**28**	**32.5%**

Breakpoints in EUCAST and CLSI

			EUCAST breakpoint	
Group	Set	Same	Lower	Higher
"S"	All, irrespective of species	95	135	37
	> 1 dilution		54	7

Comment

- EUCAST generally more conservative

  >50% of all breakpoints are lower
- When EUCAST is higher, it is mainly to avoid splitting the wild-type and thereby reduce the test error
- CLSI instead uses the "Intermediate" category to reduce test errors and prevent VME and ME.

John Turnidge, Brisbane, Australia 2015

The proportion of numerical breakpoints WITH an intermediate category in EUCAST and CLSI.

	Enterobact-eriaceae	Pseudo-monas	Staphylo-cocci	S. pneu-moniae	H. influenzae
EUCAST	56 %	41 %	43 %	64 %	39 %
CLSI	77 %	100 %	77 %	88 %	69 %

The proportion of numerical breakpoints where an intermediate category was included is higher in the CLSI than in EUCAST.
In EUCAST, each intermediate category is related to a dose or administration which is higher than the standard.

G Kahlmeter, ECCMID, Amsterdam 2016

Why the difference between breakpoints in EUCAST and CLSI?

Because most CLSI breakpoints have not been revised in a very long time!

There is no systematic review/revision process in CLSI.

Will going from CLSI to EUCAST affect AMR surveillance

Species	Resistance	Yes/No/Marginally
Entero-bacteriaceae	Ampicillin/amoxicilin	Yes (8/8 vs. 8/16 mg/L; EUCAST only S and R)
	Amoxiclav	Yes, (8/8 vs. 8/16 mg/L; and EUCAST UTI brpt 32/32 vs. CLSI 8/16 mg/L)
	Pip-tazobactam	Yes, CLSI brpt too high
	3rd gen Cephs	No (brpts the same)
	Carbapenems	Yes, EUCAST higher brpts
	Fluoroquinolones	Yes, EUCAST lower brpts
	Salmonella	No
	Aminoglycosides	No
	Colistin	No (harmonised)

Will going from CLSI to EUCAST affect AMR surveillance

Species	Resistance	Yes/No/Marginally
S. aureus	MRSA	No
	Fluoroquinolones	Marginally
	Aminoglycosides	Marginally
	Macrolides	Marginally
	Vancomycin	Yes (No intermediate)
	Linezolid	Yes, EUCAST disk better

Will going from CLSI to EUCAST affect AMR surveillance

Species	Resistance	Yes/No/Marginally
Ps. aeruginosa	Pip-tazobactam	Yes, EUCAST R-brpt lower
	Ceftazidime	Yes, EUCAST R-brpt lower
	Cefepime	Yes, EUCAST R-brpt lower
	Carbapenems	No
	Fluoroquinolones	Marginally
	Colistin	No

Will going from CLSI to EUCAST affect AMR surveillance

Species	Resistance	Yes/No/Marginally
S. pneumoniae	Penicillin-screen	No
	Penicillin V, oral	No
	Penicillin G, meningitis	No
	Penicillin, pneumoniae	Marginally
	Ampi/Amoxicillin	Yes (0.5 vs. 2 mg/L)
	Cefotaxime/ceftriaxone	No
	Erythromycin	No
	Moxifloxacin	Marginally
	Rifampicin	Yes (0.06 vs. 1.0 mg/L)

Differences in methodology?

- The basic methodology is the same.
- Both committees refer to ISO MIC broth microdilution as the reference method.
- MH-agar as a base
- the Kirby Bauer inoculum
- the same agar depth (4 mm +/-0.5 mm)
- almost the same incubation time
  EUCAST: 16-20h
  CLSI: 16-18 or 20-24h

- But there are differences in media and disks

EUCAST susceptibility testing media

- **Mueller-Hinton agar (MH)**

  Enterobacteriaceae, Pseudomonas, staphylococci and enterococci

- **Mueller-Hinton agar with 5% mechanically defibrinated horse blood and 20 mg/L β-NAD (MH-F)**

  for fastidious organisms: *S. pneumoniae* and other streptococci, Haemophilus, Moraxella, Pasteurella, Listeria, Campylobacter, Corynebacterium, Kingella, Aerococcus

Criteria from EUCAST

- We always involve media (NOT READY MADE) and disks from at least 3 manufacturers.
- We have also started to investigate the quality of commercial AST materials.
- Warnings against poor quality material are issued on the EUCAST website.

Warnings on EUCAST website

AST of bacteria

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- MIC distributions and ECOFFs
- Zone distributions and ECOFFs
- **AST of bacteria**
  - Media preparation
  - MIC determination
  - Disk diffusion methodology
  - Disk diffusion implementation
  - Compliance of manufacturers
  - Breakpoint tables
  - QC Tables
  - Calibration and validation
  - **Warnings!**

EUCAST warnings concerning antimicrobial susceptibility testing products or procedures.

The EUCAST disk diffusion development laboratories, a network of laboratories coordinated from the EUCAST development laboratory in Växjö, Sweden, from time to time discover products (disks, media batches, gradient tests or procedures) which are not performing to the expected standard. When this is the case we inform the manufacturer and publish a warning on this page.

We do not systematically test all products so the lack of a warning does not imply that there is no problem with the product in question.

Laboratories which experience problems with a susceptibility test method, and suspect that this may be related to a particular product, may contact EUCAST for advice.

1. Problems with piperacillintazobactam gradient tests from two manufacturers (see below).
2. Wide variation in disk quality in 16 disks from nine manufacturers (see below)

Checking on manufacturers

- Disks from 9 manufacturers were tested at two different time points (12 months between)
- We identifed 16 important disks
- All tests were made in triplicates and on media from two manufacturers.
- Manufacturers were informed between the two tests about their “victories and failures”

Checking on manufacturers

Jenny Åhman et al, Poster 0824, ECCMID 2016

Table 1. Evaluation of disks from nine manufacturers vs. EUCAST QC targets and ranges.**
1 = First Study, 2 = Follow-up Study

	Bio-Rad		Liofilchem		BD		Abtek		SirScan		Oxoid		HiMedia		Bioanalyse		Mast	
Antimicrobial disk	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
Benzylpenicillin 1 unit					L				H	H			NA	NA	H	H		
Amoxicillin-clav. 30 µg	H	H*					L						H	H		L		
Piperacillin-tazo. 36 µg							L	L	H				NA	NA				
Oxacillin 1 µg			L		L				L				H	H	L			
Mecillinam 10 µg							L		H				H		H			
Cefotaxime 5 µg							NA		L				NA	NA				
Cefoxitin 30 µg	H*	H*	H	H*			NA	L					L*	L*		L		
Ceftazidime 10 µg							L	L					L	H				
Meropenem 10 µg	H		H*				L	L			H		H					
Ciprofloxacin 5 µg	L				L		L	L					H	H*		L	L	
Norfloxacin 10 µg							L		L				H*	H				
Pefloxacin 5 µg			L	L	L		NA	NA	NA				H					
Gentamicin 10 µg					H		L		NA				H	H				
Tobramycin 10 µg	NA	NA	H										H*	H*				
Erythromycin 15 µg			L		L		L		L				H	H	L*	L		
Tetracycline 30 µg			L	L*	L*		L		L*					L	L		L	

**Data from the first study has been reanalyzed due to changes in QC criteria between 2015 and 2016.
These data, including information on disk lot numbers, are published on www.eucast.org.

- Mean value within ± 1 mm of the target value
- Mean value >1 mm but within ± 2 mm of the target value
- Mean value >2 mm from target value but still within the QC range
- **Mean value out of the QC range**
- Disk included in first study, but not supplied for follow-up study

NA = Not Available
H = High, mean value > 1 mm above target
L = Low, mean value > 1 mm below target
* One or more readings out of QC range

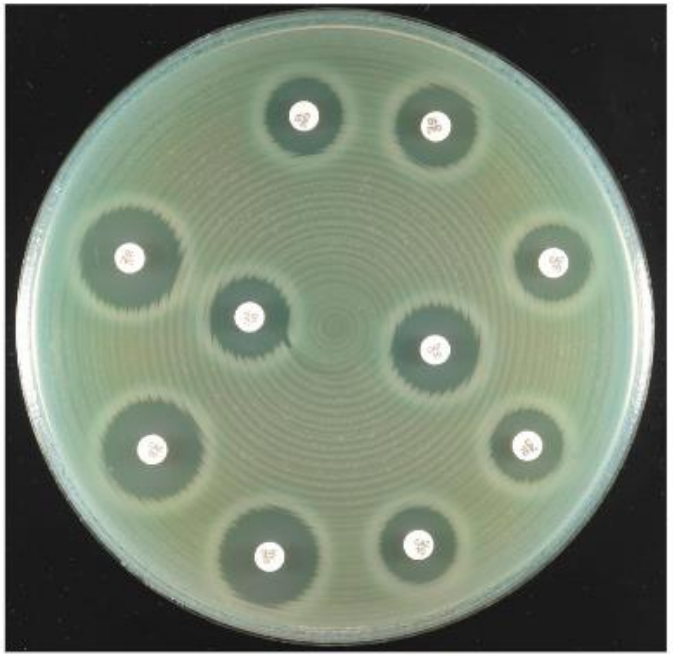
Checking on manufacturers

Jenny Åhman et al, Poster 0824, ECCMID 2016

a)

Disk manufacturer	
HiMedia	Other
16	24
18	24
16	25
15	24
16	24
20	24
20	24
21	24
16	24
21	24
16	24
16	25
16	24
18	24
17	25
20	24
20	24
20	24
15	24
15	24
Mean value	**18**	**24**
Standard deviation	***2.2***	***0.4***
Minimum value	**15**	**24**
Maximum value	**21**	**25**
EUCAST target	**24**	**24**
EUCAST range	**21-27**	**21-27**

b)



Media in EUCAST and CLSI –
MH agar for non-fastidious organisms, but…..

Species/Group	EUCAST	CLSI
Streptococcus spp.	Mueller-Hinton F	MH + 5% sheep (disk) MH + 2.5-5% LH (BMD) = ISO
Haemophilus influenzae(+para)	Mueller-Hinton F	*Haemophilus* Test Medium
Listeria monocytogenes	Mueller-Hinton F	MH + 2.5-5% LH (BMD) = ISO
M. catarrhalis	Mueller-Hinton F	MHB and MHA
Pasteurella multocida (spp.)	Mueller-Hinton F	MH + 5% sheep (disk) MH + 2.5-5% LH (BMD) = ISO
Campylobacter jejuni/coli	Mueller-Hinton F	MH + 5% sheep (disk) MH + 2.5-5% LH (BMD) = ISO

Most media are the same except…

Species/Group	EUCAST	CLSI
Corynebacterium spp.	Mueller-Hinton F	MH + 2.5-5% LH (BMD) = ISO
N. gonorrhoeae	(MIC method)	GC Agar + suppl.
N. meningitidis	(MIC method)	MH + 5% sheep (disk) MH + 2.5-5% LH (BMD) = ISO
Helicobacter pylori	(MIC method)	MH + 5% sheep aged (disk)
Anaerobes	(MIC method)	**Brucella*** + haemin + Vit K (agar dilution, add LHB for BMD)

*Brucella agar show great variation between manufacturers and can not be used in a standard method

Difference in tests available for…

Species	EUCAST v5	CLSI M100-S25
Cefazolin	No	✓
Cefoperazone-sulbactam	No	No
Cephalexin	✓	(✓ CZL - UTI)
Fosfomycin IV	✓	No
Fusidic acid	✓	No
Teicoplanin	✓	No
Telavancin	✓	✓
Tigecycline	✓	FDA only
Tetracyclines	✓	✓ unrevised
Older (uncommon) cephalosporins		(✓ unrevised)
Older (uncommon) fluoroquinolones		(✓ unrevised)
Older (uncommon) aminoglycosides		(✓ unrevised)

Differences in Disk Strengths

Agent	Species	EUCAST	CLSI
Ampicillin	*Enterococcus* spp. *H. influenzae* *P. multocida*	2µg	10µg
Amoxycillin-clavulanate	*H. influenzae* *M. catarrhalis* *P. multocida*	2-1µg	20-10µg
Piperacillin	Enterobacteriaceae *Pseudomonas* spp.	30µg	100µg
Piperacillin-tazobactam	Enterobacteriaceae *Pseudomonas* spp.	30-6µg	100-10µg
Cefotaxime	Enterobacteriaceae Viridans *Streptococcus* spp. *H. influenzae*	5µg	30µg
Ceftazidime	Enterobacteriaceae *Pseudomonas* spp.	10µg	30µg

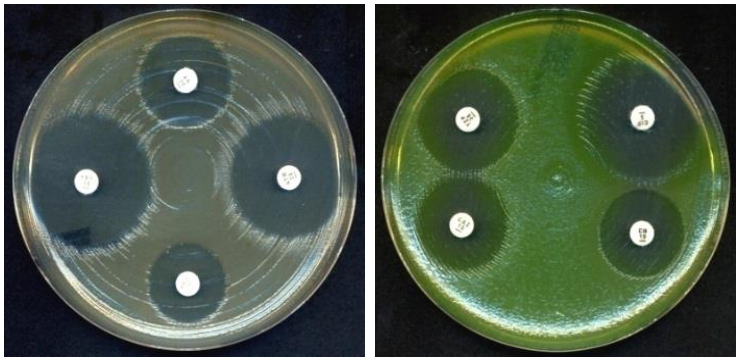
Differences in Disk Strengths

Agent	Species	EUCAST	CLSI
Ceftaroline	Enterobacteriaceae *S. aureus*	5µg	30µg
Netilmicin	Enterobacteriaceae *Staphylococcus* spp.	10µg	30µg
Benzylpenicillin	*Staphylococcus* spp. β-haem & viridans *Streptococcus* spp.	1 unit	10 units
Linezolid	*Staphylococcus* spp. *Enterococcus* spp. β-haem *Streptococcus* spp.	10µg	30µg
Nitrofurantoin	Enterobacteriaceae *Staphylococcus* spp. *Enterococcus* spp.	100µg	300µg
Vancomycin	*Enterococcus* spp. β-haem *Streptococcus* spp. *S. pneumoniae*	5µg	30µg

The 15-15-15 minute rule

- Use the inoculum within **15 minutes** of preparation
  - and always within 60 minutes
- Apply disks within **15 minutes** of inoculating plates
- Start incubation within **15 minutes** of application of disks

The growth should be confluent and evenly spread over the plate



Plates should look like this..

..and NOT like this!

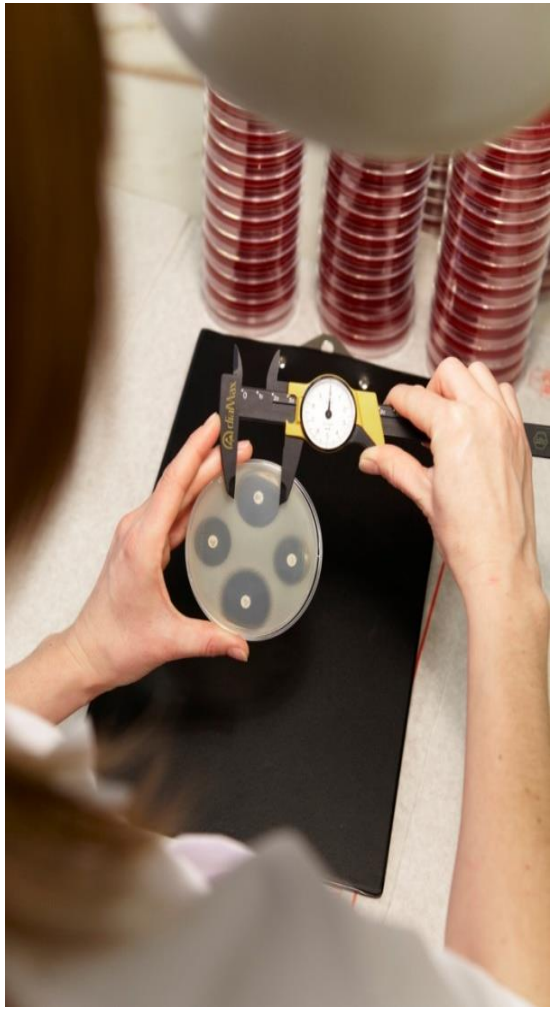
Reading of zones

- MH plates

  Read zones from the back of the plate against a dark background and illuminated with reflected light.

- MH-F plates

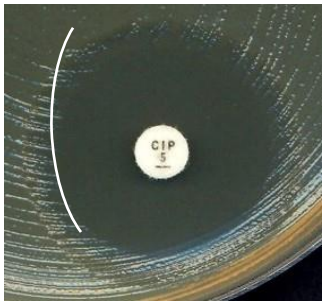
  Read zones from the front of the plate with the lid removed and illuminated with reflected light.



Reading of zones

Read zone edges at the point where no obvious growth is detected by the unaided eye with the plate held about 30 cm from the eye.

Examples:



E. coli
Ciprofloxacin

S. aureus
Erythromycin

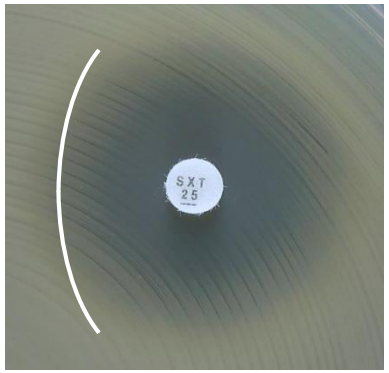
CoNS
Trimethoprim

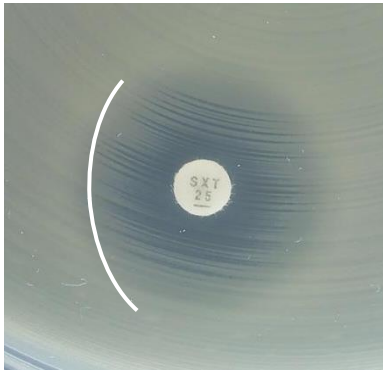
S. pneumoniae
Rifampicin

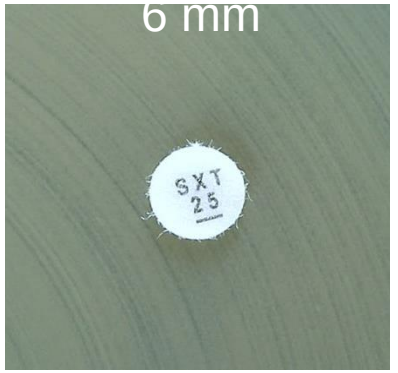
Reading guide available at www.eucast.org

Stenotrophomonas maltophilia and trimethoprim-sulfamethoxazole

- Ignore growth within the inhibition zone. The density of growth in the zone may vary from a fine haze to substantial growth.







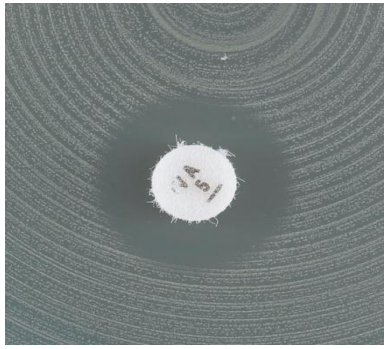
Ignore growth and read an inhibition zone if any zone edge can be seen.
= Susceptible if zone diameter ≥ 16 mm

Growth up to the disk and no sign of inhibition zone
= Resistant

Enterococci and vancomycin

- Examine with transmitted light (plate held up to light).
  - Fuzzy zone edges and colonies within zone indicate vancomycin resistance. If the zone diameter is ≥ 12 mm and the zone edge is fuzzy, investigate further.

E. faecalis
non-VRE



E. faecium VRE

Evaluation of QC results

Before implementation of EUCAST methods

- A training period of approximately 2 months

After implementation of EUCAST methods

- Perform frequent QC
  - Daily or at least four times per week
- Record inhibition zone diameters and compare inhibition zone distributions with reference distributions at the EUCAST website

QC ranges and targets

Routine QC

EUCAST QC Tables v. 6.1, valid from 2016-03-01

***Escherichia coli* ATCC 25922**

(NCTC 12241, CIP 76.24, DSM 1103, CCUG 17620, CECT 434)

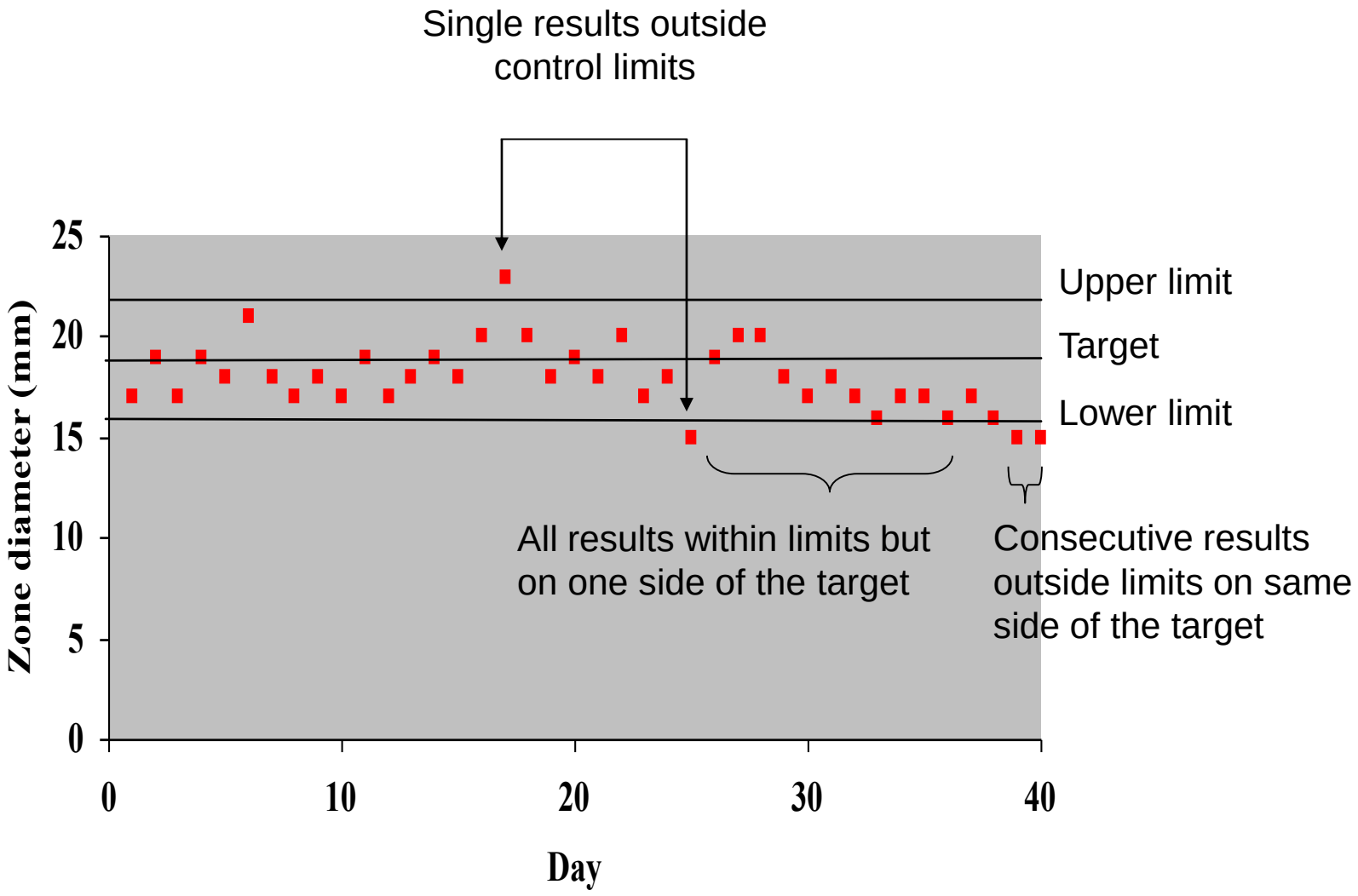
Disk diffusion methodology: Mueller-Hinton agar, McFarland 0.5, air, 35±1°C, 18±2h. Read zone edges as the point showing no growth viewed from the back of the plate against a dark background illuminated with reflected light.

Antimicrobial agent	MIC (mg/L)		Disk content (µg)	Inhibition zone diameter (mm)	
	Target[1]	Range[2]		Target[1]	Range[3]
Amikacin	1-2	0.5-4	30	22-23	19-26
Amoxicillin	4	***2-8***	-	-	-
Amoxicillin-clavulanic acid[4,5]	4	2-8	20-10	21	18-24[6]
Ampicillin	4	2-8	10	18-19	15-22[6]
Ampicillin-sulbactam[5,7]	***2***	***1-4***	10-10	21-22	19-24[6]
Aztreonam	0.125	0.06-0.25	30	32	28-36
Cefadroxil	-	-	30	***17***	***14-20***
Cefalexin	8	4-16	30	***18***	***15-21***
Cefepime	0.03-0.06	0.016-0.125	30	34	31-37
Cefixime	0.5	0.25-1	5	25	23-27
Cefotaxime	0.06	0.03-0.125	5	***28***	***25-31***
Cefoxitin	4	2-8	30	26	23-29

Range
Used to allow random variation

Target
Mean values from repeated measurements should optimally be on target ± 1 mm (mode MIC on target)

QC ranges and targets



EUCAST laboratory facilities

- EUCAST Development Laboratories
  - Bacteria - Växjö, Sweden
  - Fungi - Copenhagen, Denmark
  - Education, coordination and development
- EUCAST Network Laboratories (ca 20 globally)
  - Develop, validate and troubleshoot EUCAST methods and/or train and educate other laboratories
  - We invite laboratories to sign up – pet bug, pet drug, help develop QC ranges, etc.

EUCAST Development & Network Laboratories

Bacteria

EUCAST Development Laboratory for bacteria, Växjö, Sweden

Network Laboratories (n=14)

- Southmead Hosp., Bristol, **UK**
- Karolinska University Hosp., Stockholm**, Sweden**
- Acibadem Labmed Clinical Lab. Istanbul, **Turkey**
- Clinical microbiology, Kalmar Hosp., **Sweden**
- Aarhus Univ. Hospi., **Denmark**
- Hosp. Univ. Ramon y Cajal, Madrid, **Spain**
- analyse BioLab, Linz, **Austria**
- Haukeland University Hosp. Bergen, **Norway**
- Stavanger University Hosp. Stavanger, **Norway**
- Univ. of Verona, **Italy**
- Provincial Lab. for Public Health Alberta, **Canada**
- University Hospital of North N**orway**
- Ist. Zooprofilattico Sperimentale, Sassari, **Italy**
- Vestfold Hospital Trust, Tønsberg, **Norway**

Fungi

EUCAST Development Laboratory for fungi, SSI, Copenhagen, Denmark

Network Laboratories (n=11)

- Spanish Mycology Ref. Loratory, **Spain**
- Hopital Européen Georges Pompidou, **France**
- Gregorio Marañón Hosp., Madrid, **Spain**
- National Ref. Centre Invasive Mycoses, **Germany**
- Clinical Microbiology Lab. Athens, **Greece**
- Mycology Reference Centre, Manchester, **UK**
- Erasmus MC, **The Netherlands**
- University of Athens, **Greece**
- Radboud MC, **The Netherlands**
- Medical University of Innsbruck, **Austria**
- Lab. Antimicrobial Chemotherapy, **Romania**

Increasing international reach

- More countries outside Europe are going over to EUCAST.
- Website hits now >50 000 per month.
- User questions from all over the world are increasing.
- >90% of labs in Queensland, Australia, are now on EUCAST.
- Almost 90% of NEQAS subscribers are now on EUCAST.
- All “competitors” (except CLSI) have resigned and joined EUCAST.

EUCAST leadership

Chair

- Ian Philips 1997 – 2001
- Gunnar Kahlmeter 2001 – 2012
- Rafael Canton 2012 – 2016
- Christian Giske 2016 –

Scientific secretary

- Derek Brown 1997 – 2016
- John Turnidge 2016 -

EUCAST Steering Committee today

- Christian Giske, chair
- Derek Brown, scientific secretary
- Rafael Canton, clinical data coordinator
- Gunnar Kahlmeter, technical data coordinator/webmaster
- Sören Gaterman, Germany
- Christoffer Lindemann, Norway
- Johan Mouton, The Netherlands
- Alasdair MacGowan, UK
- Gerard Lina, France
- Arjana Tambic, Croatia
- Deniz Gur, Turkey

Acknowledgements

To all those who told me it could never be done!

And to all those who believed it could happen!

South Africa May 2016

South Africa May 2016


South Africa May 2016

South Africa May 2016

One of 23 NCCLS/CLSI meetings

Somewhere along the line I was promoted to first gunner!

Thank you

Phenotypic susceptibility testing is based on


MIC
MIC
MIC
MIC
MIC

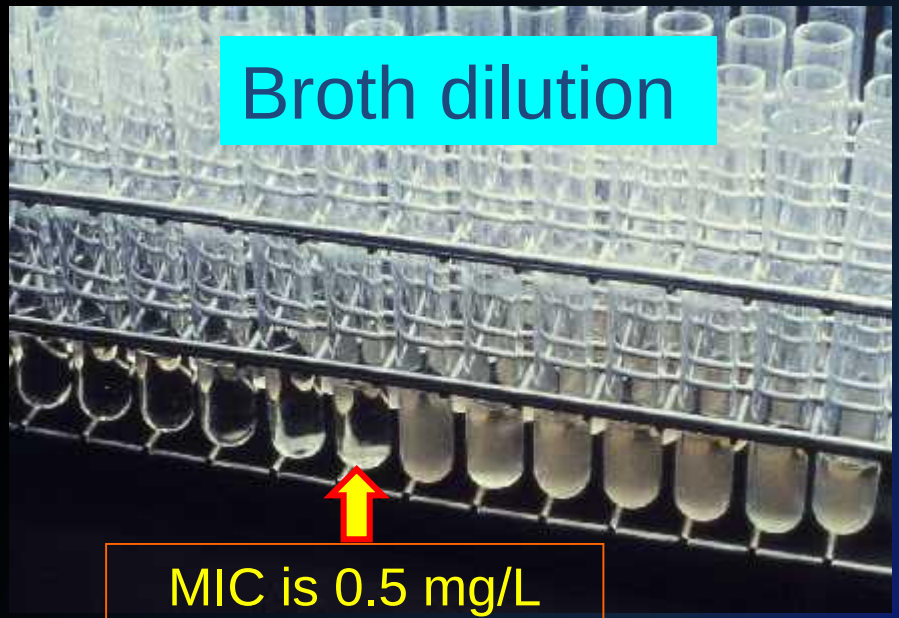

akpoints
MIC
Breakpoint
Breakpoi
MIC
MIBreakpoint
Breakpoints

MIC

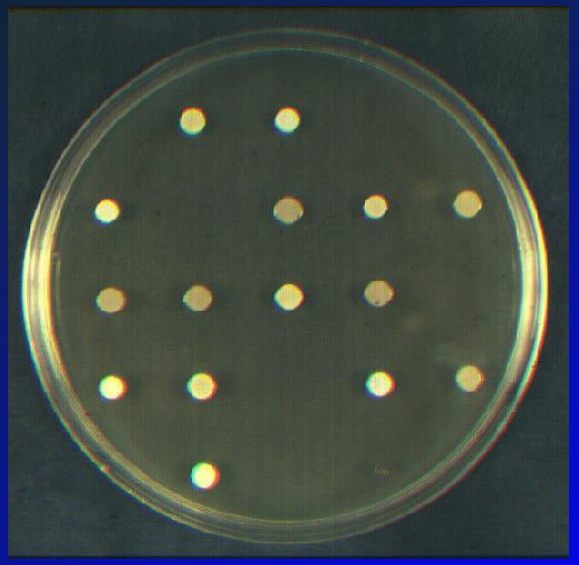
is a relative measure

- Influenced by pH, cations …
- Influenced by inoculum
- Influenced by incubation time
- Influenced by temperature
- Discontinuous variable
- Is often misinterpreted as “absolute”

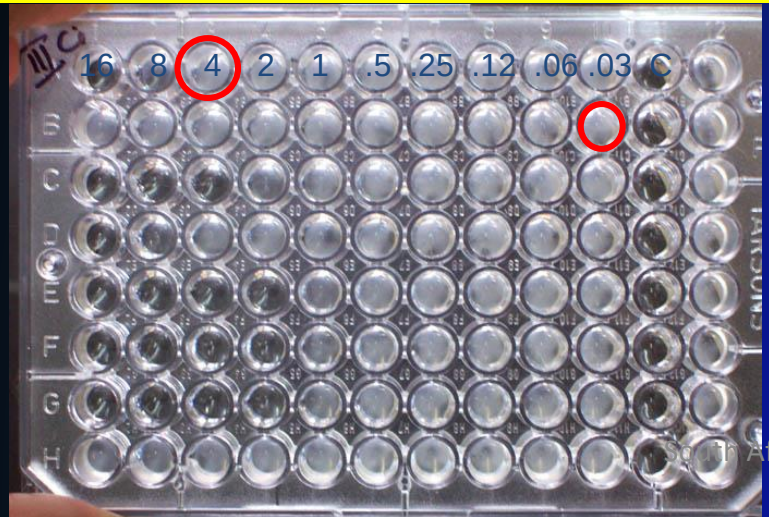
Methods for MIC determination



Agar dilution



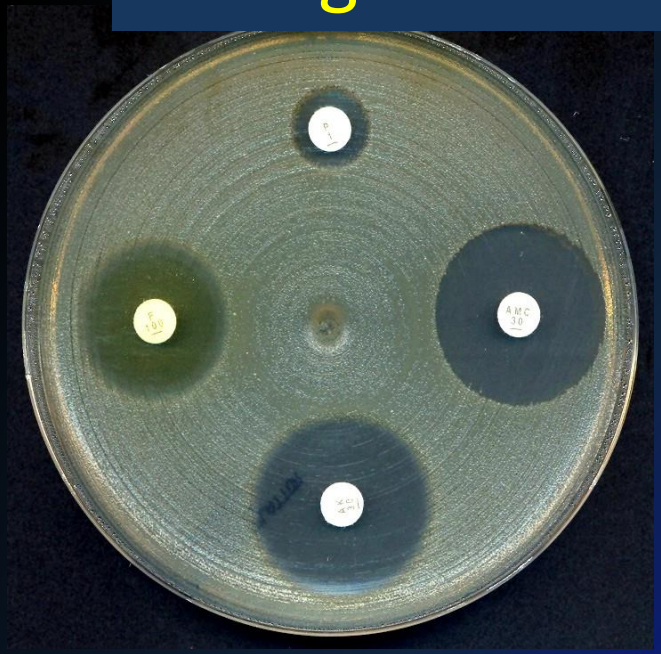
Broth microdilution (BMD)

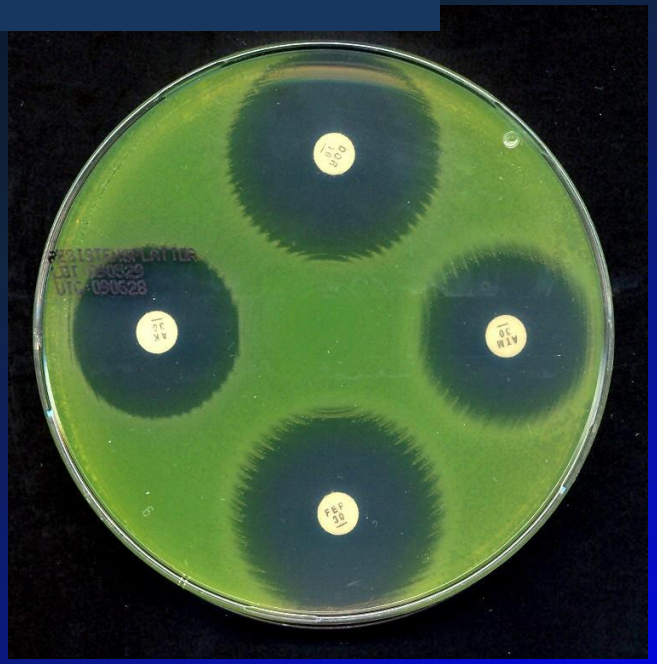


Gradient MIC test

Several manufacturers:
bioMerieux (Etest)
Oxoid (M.I.C.E.)
Liofilchem (MIC-strip)

Surrogate MIC determination

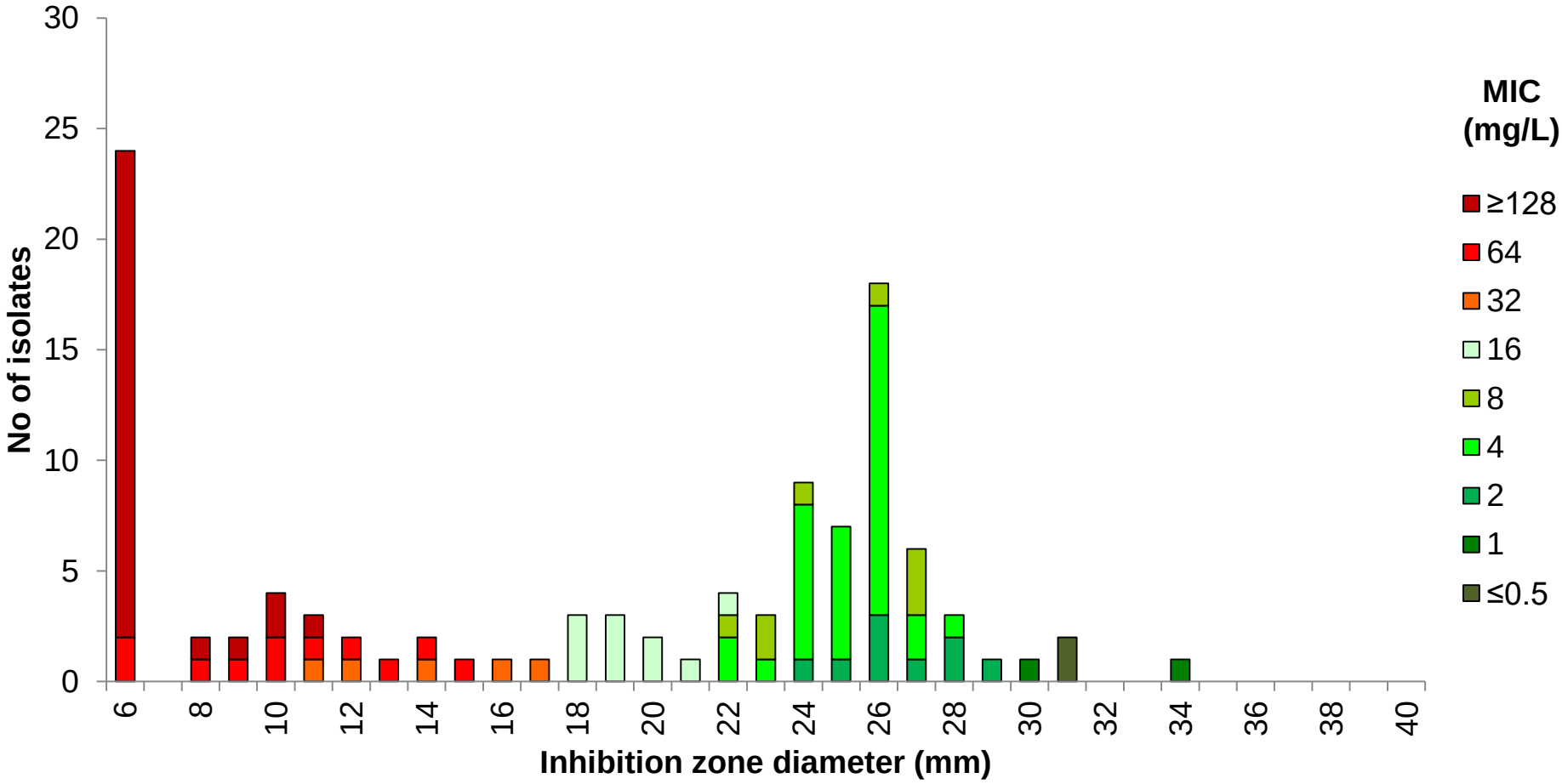


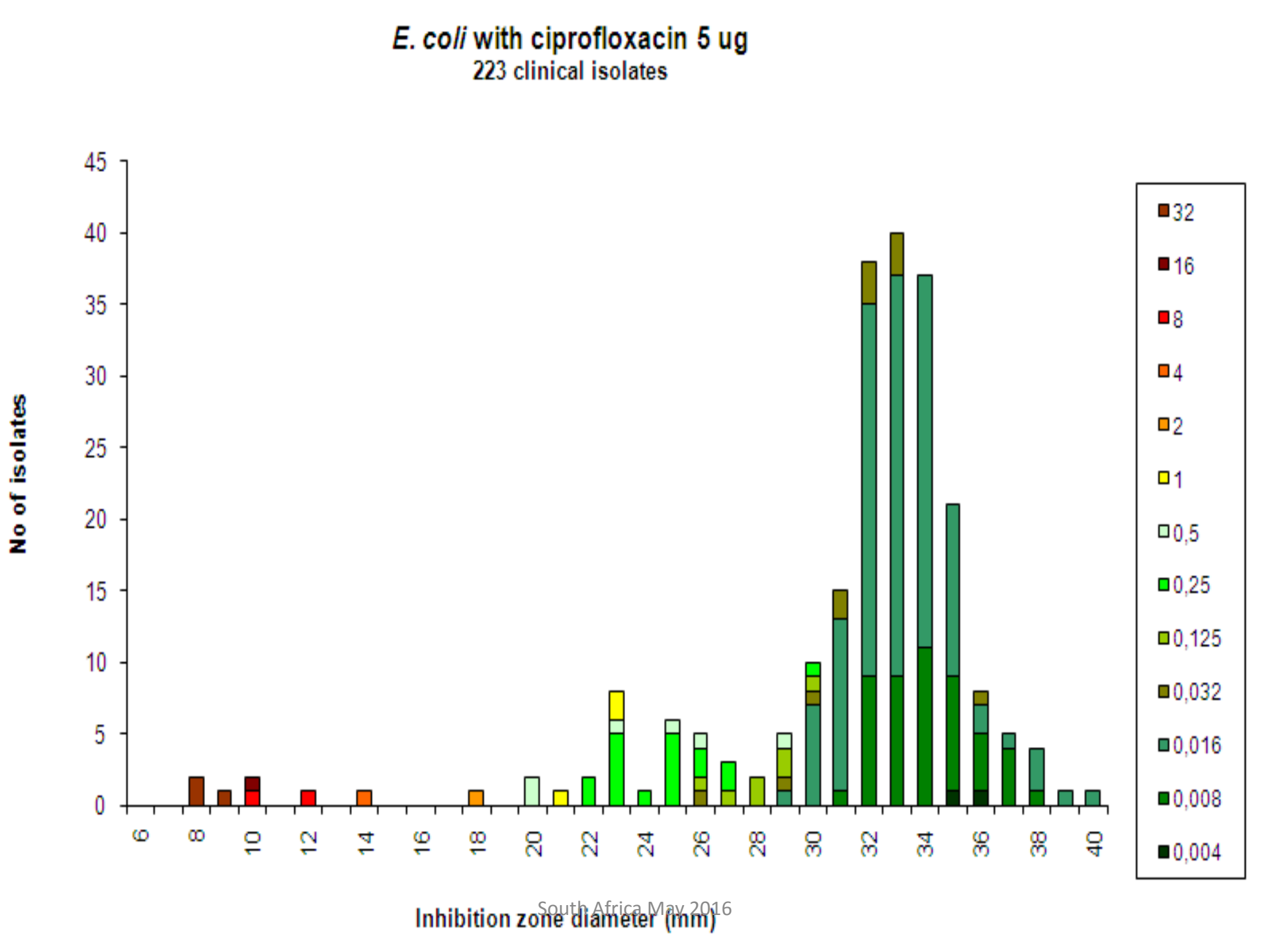


…and when disk diffusion is well standardised the correlation between MIC and zone diameter is (with some exceptions) very good…

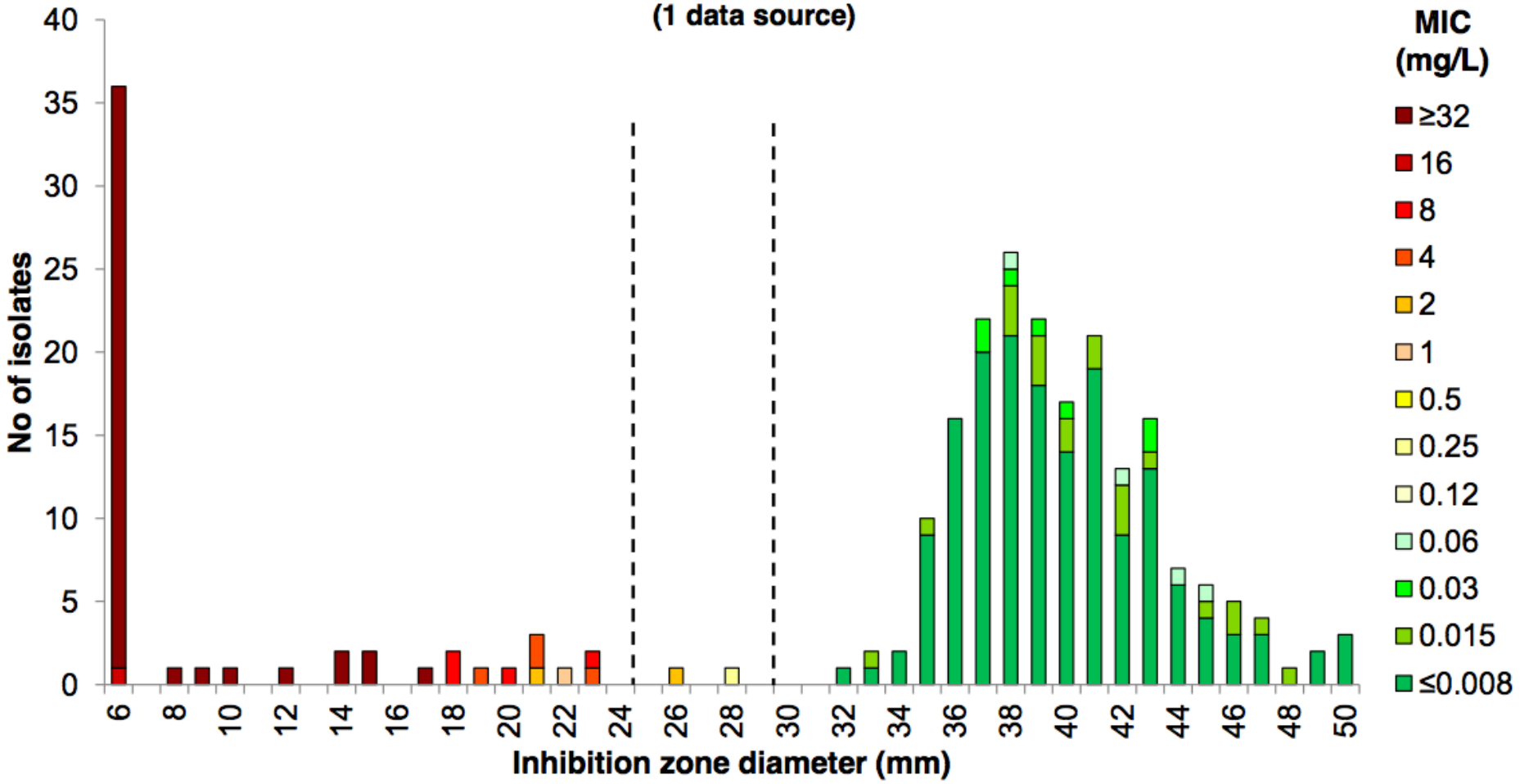
…whereas calibrating gradient tests for the whole scale of IC values is quite tricky…

Pseudomonas aeruginosa, 107 clinical isolates
Piperacillin-tazobactam 30-6 µg vs. MIC




E. coli with ciprofloxacin 5 ug
223 clinical isolates
No of isolates
45
40
35
30
25
20
15
10
5
0
6
8
10
12
14
16
18
20
22
24
26
28
30
32
34
36
38
40
Inhibition zone diameter (mm)
32
16
8
4
2
1
0,5
0,25
0,125
0,032
0,016
0,008
0,004

Rifampicin 5 *µ*g vs. MIC
Corynebacterium spp., 253 clinical isolates



Breakpoints

MIC	S≤0.06, R>0.5 mg/L
Zone diameter	S≥30, R<25 mm

Milestones in the development of AST

- Beijerinck in 1889 used agar diffusion to study the effect of different auxins (plant growth hormones) on bacterial growth.
- Fleming in 1924 introduced the use of the ditch plate technique for evaluating antimicrobial qualities of antiseptic solutions.
- Fleming later developed a broth dilution technique with turbidity as end point.
- The WHO commissioned the ICS published in 1971 (Ericsson and Sherris) – but Garrod was less than enthusiastic
- The 1970ies - the formation of national breakpoint committees (DIN, NCCLS, and others).
- EUCAST formed 1997 and reorganised 2001
- ISO 20776-1 (2006) – International reference for broth microdilution MIC determination in non-fastidious bacteria.
- 2016 ISO 200776 - revised

Hans Ericsson
(Sweden)

John Sherris
(USA)

Antibiotic Sensitivity Testing

Report of an International Collaborative Study

BY

HANS M. ERICSSON and JOHN C. SHERRIS

MUNKSGAARD. COPENHAGEN

Courtesy Ron Jones

Ericsson, Sherris and WHO were critizised for recommending rigorous standardisation

- Balows, head of CDC 1972, commenting on the ICS approach, Balows deemed it impractical and too demanding. It also implied a level of standardisation that might result in violation of property rights: 'I doubt seriously that commercial concerns would willingly or should even be expected to describe or reveal their procedures for impregnation and drying [of discs]. In the USA this might well be regarded as an infringement of their proprietary procedures …

- Garrod: "I must explain that although I took some part in the International Collaborative Study I have for several years disagreed with the direction it was leading.
" The ICS demands a degree of standardisation of the culture medium and of other features of the test, which I believe to be impracticle".
Somewhat later, Garrod sharpened his critique: '…the I.C.S. method is essentially that which has been advocated for years by professor Ericsson ...'

- Germany: A national committee on sensitivity testing had voiced concerns in September 1963 that some of Ericsson approaches were 'too complicated given conditions in German laboratories; it seems possible to implement simplifications without compromising precision'.

….these and similar arguments are reiterated throughout the following 50 years!

- "…we cannot have different breakpoints for different species…."
- "…is it reasonable to ask laboratories to speciate gramnegatives?"
- "…we cannot put our recommendations on the internet – only few laboratories will have access…"
- "…laboratories do not distinguish between *E. fecalis* and *E. fecium* – breakpoints must be the same!"
- "…very few laboratories will ever afford a masspec.."
- "…laboratories are not staffed to cope with the extra workload of measuring zone diameters…"

It is now 40 years later and much more complicated than anything suggested by the ICS and Ericsson and Sherris.

In the beginning there was one table for everything - one MIC breakpoint and one zone diameter breakpoint to fit all.

TABLE 2. ***Zone Diameter Interpretive Standards and Approximate Minimum Inhibitory Concentration (MIC) Correlates***

Antimicrobial Agent	Disc Content	Zone Diameter, nearest whole mm			Approximate MIC Correlates[a]	
		Resistant	Intermediate[q]	Susceptible	Resistant	Susceptible
Amikacin[b]	30 μg	≤ 14	15–16	≥ 17	≥ 32 μg/mL	≤ 16 μg/mL
Ampicillin[c] when testing gram-negative enteric organisms and enterococci	10 μg	≤ 11	12–13	≥ 14	≥ 32 μg/mL	≤ 8 μg/mL
Ampicillin[c] when testing staphylococci[d] and penicillin G-susceptible microorganisms	10 μg	≤ 20	21–28	≥ 29	β-lactamase[d]	**≤ 0.25 μg/mL**
Ampicillin[c] when testing *Haemophilus* species[e]	10 μg	≤ 19	—	≥ 20	**≥ 4 μg/mL**	≤ 2 μg/mL
Bacitracin	10 units	≤ 8	9–12	≥ 13	—	—
Carbenicillin when testing the ***Enterobacteriaceae***	100 μg	≤ 17	18–22	≥ 23	≥ 32 μg/mL	≤ 16 μg/mL
Carbenicillin when testing *Pseudomonas aeruginosa*	100 μg	≤ 13	14–16	≥ 17	**≥ 256 μg/mL**	**≤ 128 μg/mL**
Cefamandole[f]	30 μg	≤ 14	15–17	≥ 18	≥ 32 μg/mL	**≤ 8 μg/mL**
Cefotaxime[f]	**30 μg**	**≤ 14**	**15–22[q]**	**≥ 23**	**≥ 64 μg/mL**	**≤ 8 μg/mL**
Cefoxitin[f]	30 μg	≤ 14	15–17	≥ 18	≥ 32 μg/mL	**≤ 8 μg/mL**
Cephalothin[g]	30 μg	≤ 14	15–17	≥ 18	≥ 32 μg/mL	**≤ 8 μg/mL**
Chloramphenicol	30 μg	≤ 12	13–17	≥ 18	≥ 25 μg/mL	≤ 12.5 μg/mL
Clindamycin[h]	2 μg	≤ 14	15–16	≥ 17	≥ 2 μg/mL	≤ 1 μg/mL
Colistin[i]	10 μg	≤ 8	9–10	≥ 11	≥ 4 μg/mL	**j**
Erythromycin	15 μg	≤ 13	14–17	≥ 18	≥ 8 μg/mL	≤ 2 μg/mL
Gentamicin[b]	10 μg	≤ 12	13–14	≥ 15	≥ 8 μg/mL	≤ 4 μg/mL
Kanamycin	30 μg	≤ 13	14–17	≥ 18	≥ 25 μg/mL	≤ 6 μg/mL
Methicillin[k]	5 μg	≤ 9	10–13	≥ 14	**≥ 16 μg/mL**	**≤ 4 μg/mL**
Nafcillin[k]	**1 μg**	**≤ 10**	**11–12**	**≥ 13**	**≥ 8 μg/mL**	**≤ 2 μg/mL**
Nalidixic Acid[l]	30 μg	≤ 13	14–18	≥ 19	≥ 32 μg/mL	≤ 12 μg/mL
Neomycin	30 μg	≤ 12	13–16	≥ 17	—	—
Nitrofurantoin[l]	300 μg	≤ 14	15–16	≥ 17	≥ 100 μg/mL	≤ 25 μg/mL
Oxacillin[k]	**1 μg**	**≤ 10**	**11–12**	**≥ 13**	**≥ 8 μg/mL**	**≤ 2 μg/mL**
Penicillin G when testing ***staphylococci[m]***	10 units	≤ 20	21–28	≥ 29	β-lactamase[d]	≤ 0.1 μg/mL
Penicillin G when testing other microorganisms[n]	10 units	≤ 11				

NCCLS First Supplement, 1981

- "useful for anything that would grow"

Today…

- The tools used to determine clinical breakpoints are universal
- Species specific/related breakpoints when possible
- the ambition is to report not only the MIC but also an interpretation (S, I and R)

EUCAST

EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING

European Society of Clinical Microbiology and Infectious Diseases

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search term Search

QUICK NAVIGATION

The European Committee on Antimicrobial Susceptibility Testing - EUCAST

EUCAST is a standing committee jointly organized by ESCMID, ECDC and European national breakpoint committees. EUCAST deals with breakpoints and technical aspects of phenotypic in vitro antimicrobial susceptibility testing and functions as the breakpoint committee of EMA and ECDC. EUCAST does not deal with antibiotic policies, surveillance or containment of resistance or infection control. The Steering Committee is the decision making body. It is supported by a General Committee with representatives from European and other countries, FESCI and ISC. The Steering Committee also consults on EUCAST proposals with experts within the fields of infectious diseases and microbiology, pharmaceutical companies and susceptibility testing device manufacturers.

EUCAST has a subcommittee on antifungal susceptibility testing and on methods for detection of resistance mechanisms of clinical and/or epidemiological importance.

Subcommittees on expert rules for antimicrobial susceptibility testing and antimicrobial susceptibility testing of anaerobes have completed their tasks and have been disbanded.

EUCAST News

14 Mar 2014
EUCAST Disk Diffusion Method published

13 Mar 2014
ESCMID Post Graduate Course

09 Mar 2014
EUCAST Steering Committee positions for 2014 - 2016

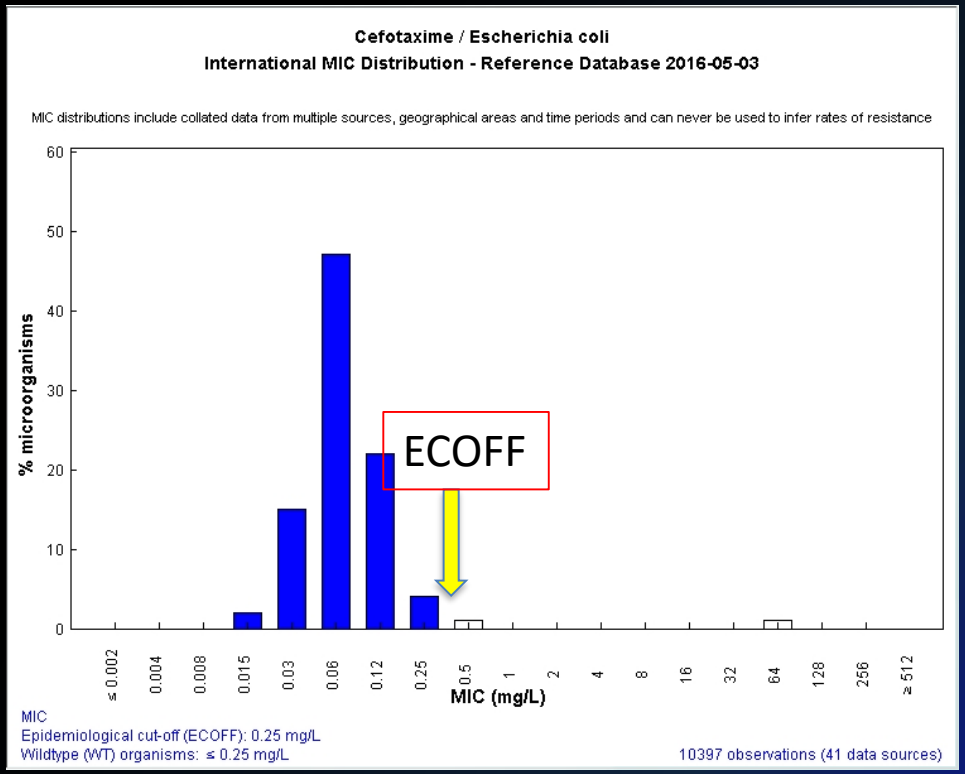
26 Feb 2014
FAQ - updated 2014-02-28

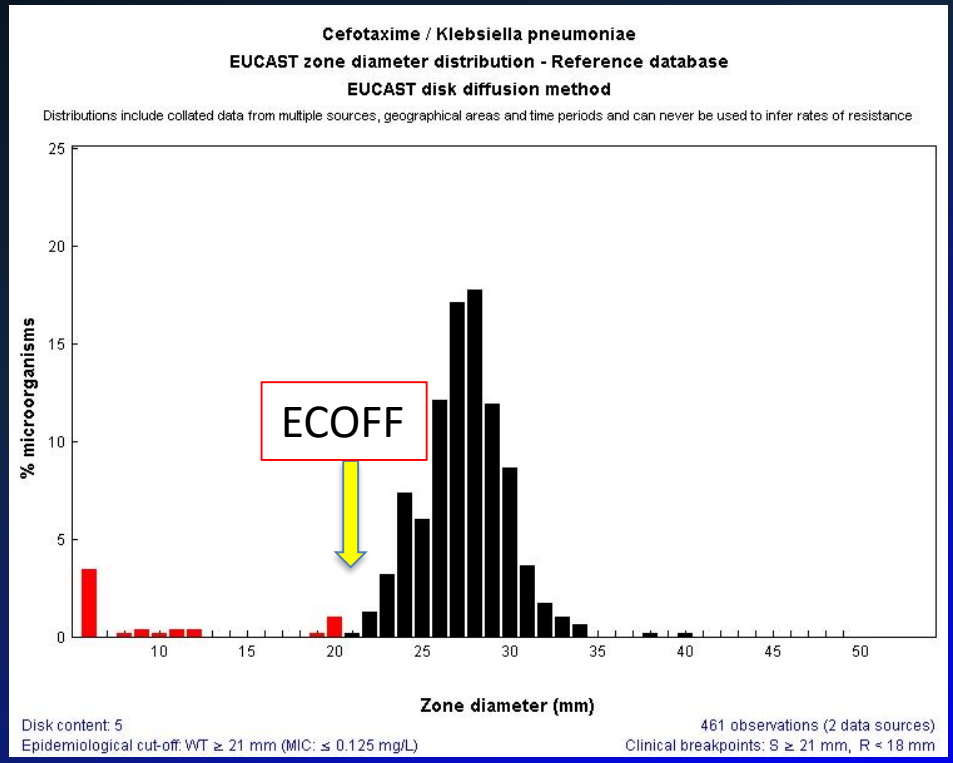
27 Jan 2014
Danish NAC presented

www.eucast.org

MIC distributions and ECOFFs on EUCAST website

- >28 000 MIC distributions
- Up to 100 000 MIC-values per distribution
- Data from many investigators (1 – 100 per distribution)
- Data from many time periods (1950 -)
- Data from many geographical areas and projects (USA, Europe, Australia, Far East, South America, Sentry, Mystic, etc)
- Data from many origins (Human clinical data, Surveillance programs, Veterinarian data, Wild life, Food safety programs)
- Ownership and responsibility:
  - Software and administration: ESCMID/EUCAST
  - Database: individual contributors own their contributions



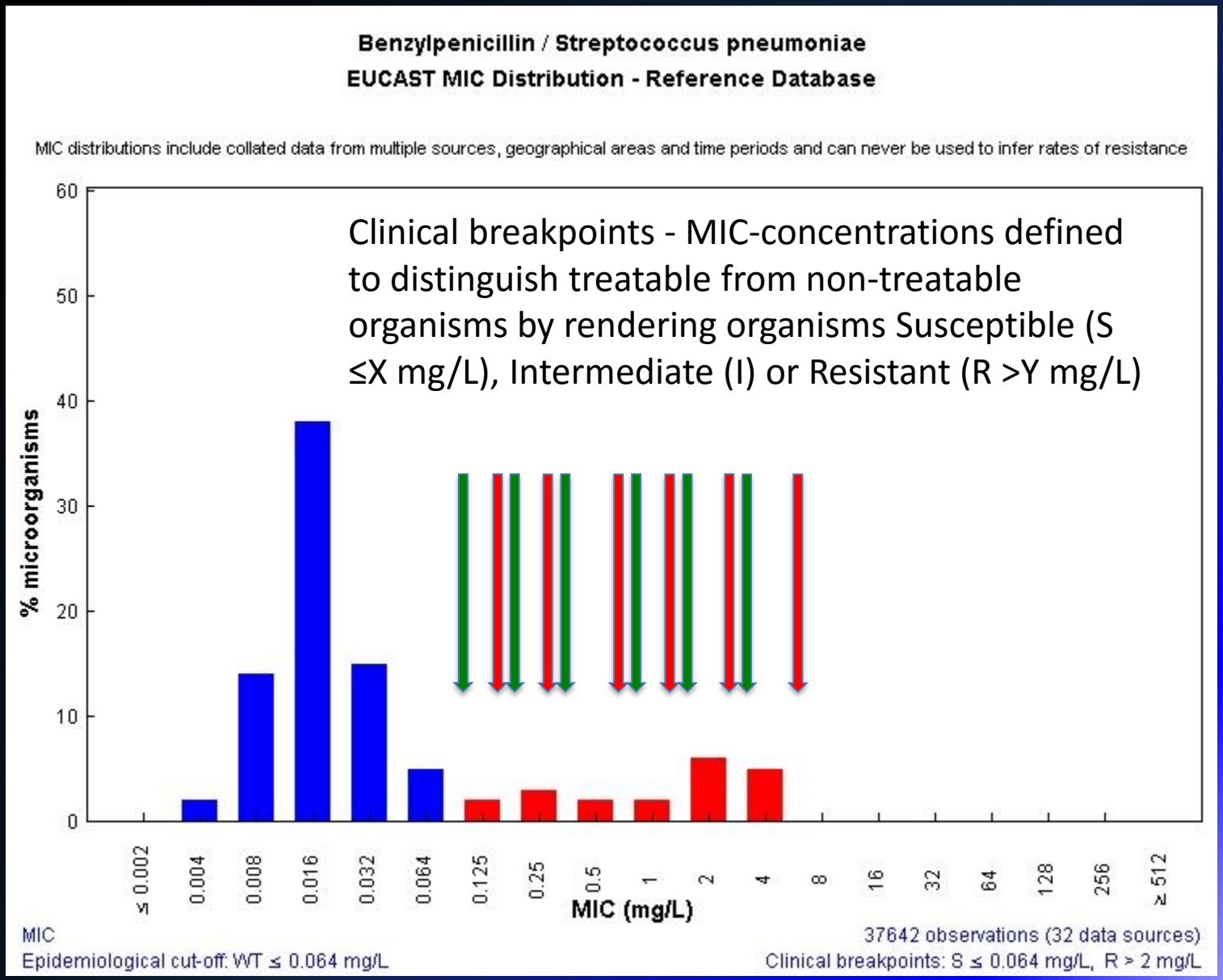


- ECOFF is the most sensitive measure of phenotypically detectable resistance.
- Within a species, it is the highest MIC of organisms lacking phenotypically expressed resistance
- If wild type organisms are considered susceptible (treatable), the ECOFF is the lowest possible S-breakpoint

Establishing ECOFFs

- A EUCAST Subcommittee is currently (2016 -) determining rules for including MIC distributions and for determining ECOFFs

Clinical breakpoints


Benzylpenicillin / Streptococcus pneumoniae
EUCAST MIC Distribution - Reference Database
MIC distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance
Clinical breakpoints - MIC-concentrations defined to distinguish treatable from non-treatable organisms by rendering organisms Susceptible (S ≤X mg/L), Intermediate (I) or Resistant (R >Y mg/L)
% microorganisms
0
10
20
30
40
50
60
≤ 0.002
0.004
0.008
0.016
0.032
0.064
0.125
0.25
0.5
1
2
4
8
16
32
64
128
256
≥ 512
MIC (mg/L)
MIC
Epidemiological cut-off: WT ≤ 0.064 mg/L
37642 observations (32 data sources)
Clinical breakpoints: S ≤ 0.064 mg/L, R > 2 mg/L

Susceptibility Testing Categorisation

Clinical breakpoints:

S≤X mg/L R>Ymg/L

S≥X mm R<Y mm

Epidemiological cutoffs (ECOFF)

WT≤X mg/L NWT>Y mg/L

WT≥X mm NWT<Y mm

Wild Type & non-Wild Type

Tools for determining clinical breakpoints

- Dose and mode of administration
- Clinical targets (indications)
- Target organisms (indications)
- MIC distributions and ECOFFs of target organisms
- Resistance mechanisms of clinical relevance in target organisms
- Pharmacokinetics of agent in target patients
- Pharmacodynamics of agent in relation to dose, infection and target organism
- Clinical outcome data for target infections

Breakpoints are determined by:

1. Medicines agencies
2. Breakpoint committees

Pharmaceutical companies
AST companies

Colleagues who know better

1. Breakpoints by Medicines agencies

(as part of the process for the approval of new drugs)

- Evaluation is based of the claims of the company
- Evaluation performed by different experts/rapporteurs for different agents.
- Agents within a group are dealt with individually and in sequence with years in between.
- “No corporate memory”.
- No systematic review process.

2. Breakpoints by Breakpoint committees

- Committee members with many competences
  - in EUCAST there are 90 experts in national groups + many external expert committees.
- When a new agent is evaluated, existing related agents are reviewed as part of the process.
- Consistency over time – “corporate” memory.
- Breakpoint committees can decide to review, and when relevant, revise breakpoints independantly of pharmaceutical companies or agencies.

Breakpoint committees

Most often these were originally technical committees

DIN (G Linzenmeier)	Germany	1973?
NCCLS (later CLSI) (A Barry)	USA	1975
NWGA (K Mellby)	Norway	1978
SRGA (RAF) (LO Kallings)	Sweden	1979
CA-SFM (Y Chabbert)	France	1980
WRG (later CRG) (P Mouton)	The NL	1981
BSAC WP on AST (I Phillips)	The UK	1988

Breakpoint committees 1970 - 2001

Committee	Country	Disc diffusion
BSAC	United Kingdom	Yes
CA-SFM	France	Yes
CRG	The Netherlands	No
DIN	Germany	Yes
NWGA	Norway	No
SRGA	Sweden	Yes
NCCLS (CLSI)	USA	Yes

Enterobacteriaceae 1975 – 2001

Committee	Amoxicillin	Cefotaxime	Piperacillin-tazob.
BSAC (UK)	8 / 16	2 / 2	16 / 16
CA-SFM (F)	4 / 16	4 / 32	8 / 64
CRG (NL)	2 / 16	4 / 8	0.25 / 4
DIN (D)	2 / 8	2 / 8	0.12 / 1
NCCLS (USA)	8 / 16	8 / 32	16 / 64
NWGA (N)	0.5 / 8	1 / 2	8 / 16
SRGA (S)	1 / 8	0.5 / 1	16 / 16

All of us managed to come up with different breakpoints.

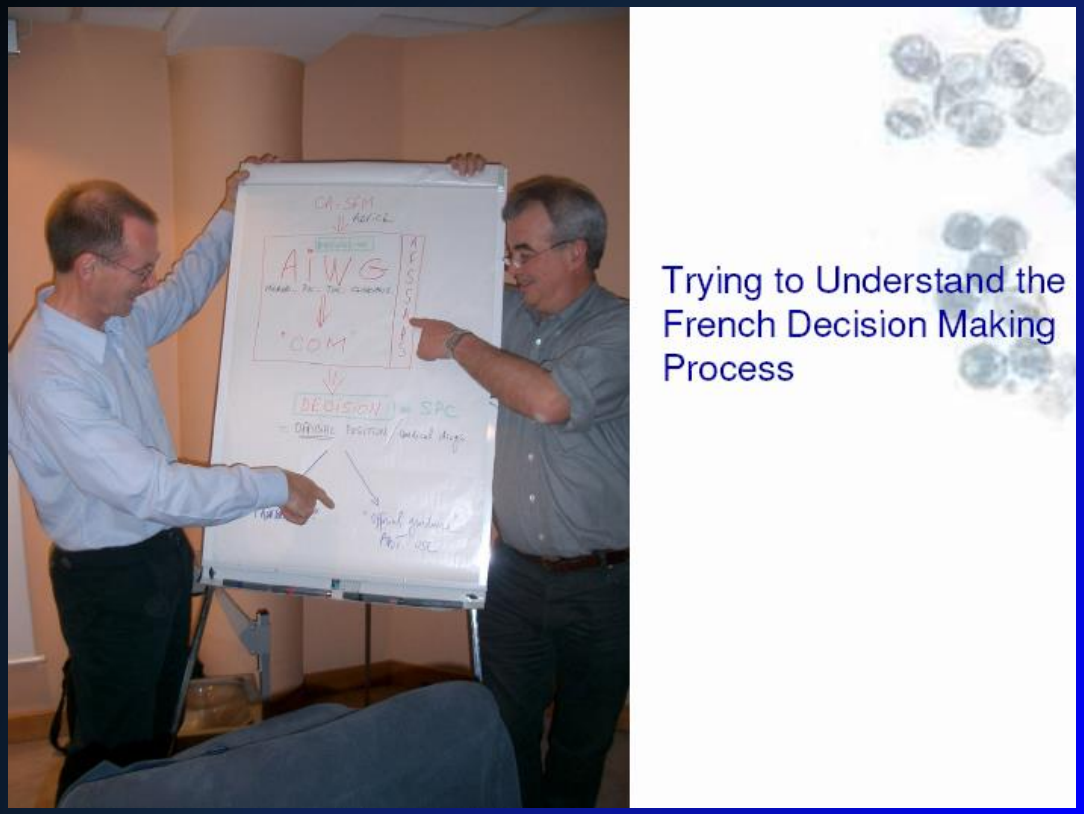
The breakpoint committees did not agree…

- …not because we disagreed
- …but we were out of sync
- …and did not communicate with each other
- …and we all knew best

EUCAST 1997 - 2001

- EUCAST was formed in 1997
- ESCMID decision and funding
- Ian Phillips was its first chairman
- Derek Brown its first Scientific secretary
- It produced a several discussion documents and breakpoints on Linezolid but ESCMID questioned its usefulness.
- In 2001 Ian was resigning and I was asked by ESCMID to evaluate the viability of EUCAST.

EUCAST was reformed in 2001/2
Responsibility for a European system was given to the 6 national breakpoint committees



I was lucky to convince Derek Brown to continue as the scientific secretary of EUCAST.


South Africa May 2016

Decision process

- Steering Committee takes preliminary decision
- For new agents, decisions are between EUCAST and EMA with input from the company.
- All other major decisions are for open consultation via EUCAST webpage
- Open consultation, rebuttals and final SC decision are published.

Breakpoints in EUCAST

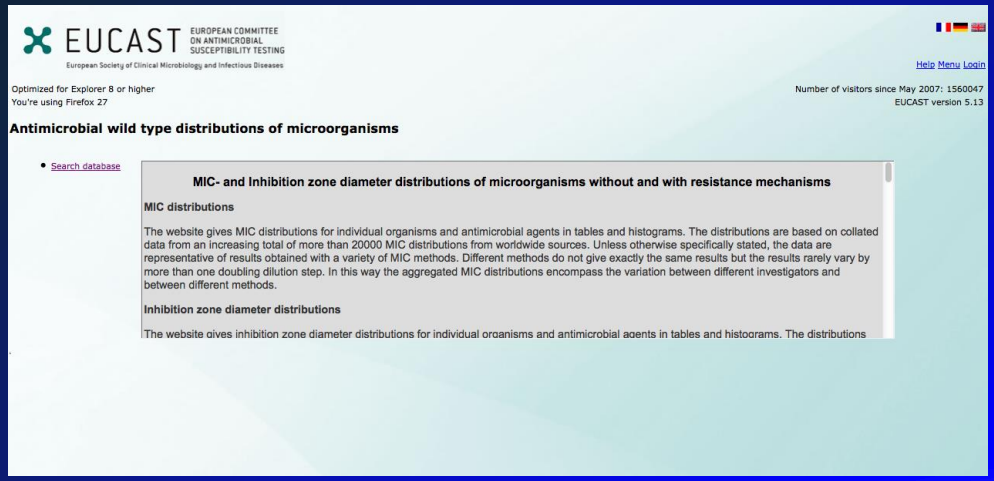
- Existing agents - harmonization of European breakpoints (2002 – 2008) for antibiotics commonly used and available in most countries:

  Penicillins, cephalosporins, carbapenems, aminoglycosides, fluoroquinolones, tetracyclines, glycopeptides, macrolides etc

- New agents - together with EMA (2003 -)
  - Daptomycin
  - Tigecycline
  - Doripenem
  - Telavancin, Oritavancin, Dalbavancin
  - Ceftaroline
  - Bedaquiline
  - Ceftobiprole
  - Tedizolid
- Review of established breakpoints (2009 -): Glycopeptides, Carbapenems, Colistin, Tigecycline, Fluoroquinolones ….

EUCAST Websites
free access

EUCAST General website



The EUCAST MIC and zone diameter distribution website

www.eucast.org

Guidance on reading EUCAST Breakpoint Tables

EUCAST Clinical Breakpoint Tables v. 6.0, valid from 2016-01-01

The intermediate category is not listed but is interpreted as the values between the S and the R breakpoints. If the S and R breakpoints are the same value there is no intermediate category.

Agent A: No intermediate category
Agent B: Intermediate category: 4 mg/L, 23-25 mm
Agent G: Intermediate category: 1-2 mg/L, 24-29 mm

Disk diffusion (EUCAST standardised disk diffusion method)
Medium:
Inoculum:
Incubation:
Reading:
Quality control:

EUCAST method for antimicrobial susceptibility testing by disk diffusion and recommendations for quality control

Breakpoints with a species name apply only to that particular species (in this example *S. aureus*)

Antimicrobial agent	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Antimicrobial agent A	1[1]	1[1]	X	20[A]	20[A]	1. Comment on MIC breakpoints
Antimicrobial agent B, *S. aureus*	2[2]	4	Y	26	23	2. New comment
Antimicrobial agent C	IE	IE		IE	IE	~~Removed comment~~
Antimicrobial agent D	-	-		-	-	A. Comment on disk diffusion
Antimicrobial agent E	IP	IP		IP	IP	
Antimicrobial agent F (screen)	NA	NA	Y	25	25	
Antimicrobial agent G	0.5	2	Z	30	24	

Changes from previous version highlighted in yellow

Screening breakpoint to differentiate between isolates without and with resistance mechanisms

Not Applicable

In Preparation

No breakpoints. Susceptibility testing is not recommended

MIC breakpoints in blue are linked to MIC distributions

Zone diameter breakpoints in blue are linked to zone diameter distributions

Antimicrobial agents in blue are linked to EUCAST rationale documents

Insufficient evidence that the organism or group is a good target for therapy with the agent

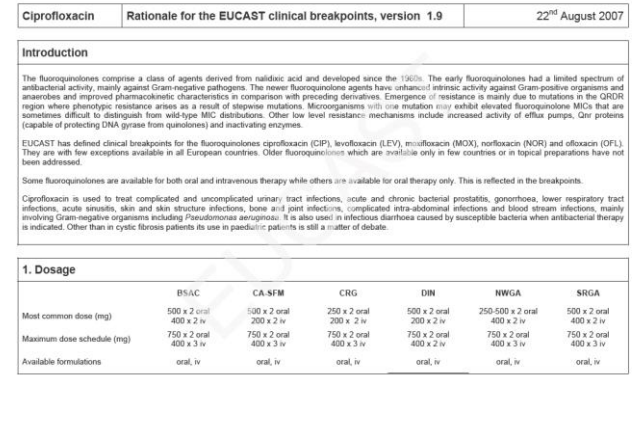
Links in EUCAST breakpoint table

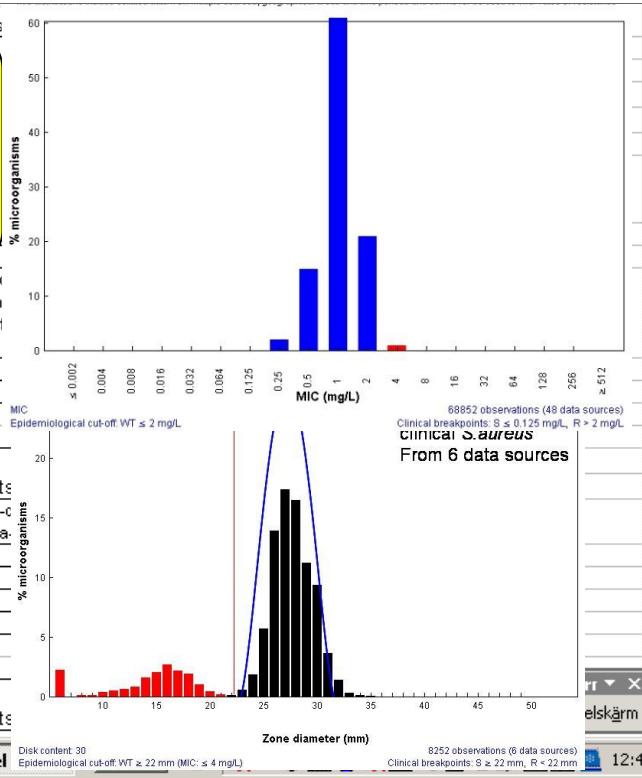
Click on antibiotic for **Rationale Document**

Click on MIC breakpoint for **MIC distributions**

Click on zone breakpoint for **zone diameter distributions**

	A	B	C	D	E	F
44						
45	**Carbapenems**					
46						
47						
48	Doripenem					
49	Ertapenem					
50	Imipenem[1]					
51	Meropenem	2	8	**10**	22	16
52						
53						
54	**Monobactams**	**MIC breakpoint**		**Disk**	**Zone diameter**		**Notes**
55		**S ≤**	**R>**	**content**	**S ≥**	**R<**	Numbers for comments on MIC bre
56							
57	Aztreonam[1]	1	8	**30**	25	21	**1**. The aztreonam breakpoints for Enterob clinically important beta-lactamases in Ente susceptible or intermediate with these breakpoints may want to use a test which specifically screens
58							
59							
60	**Fluoroquinolones**	**MIC breakpoint**		**Dis**			
61		**S ≤**	**R>**	**cont**			
62							
63	Ciprofloxacin[1]	0.5	1	5			
64	Levofloxacin	1	2	5			
65	Moxifloxacin	0.5	1	5			
66	**Halidixic acid (screen)**	Note[2]	Note[2]	**30**	16*	16*	**2/A**. Nalidixic acid may be used to screen for fluor breakpoint correlates to an MIC value of 16 mg/L in resistant to all fluoroquinolones. If other Enterobact
67	Norfloxacin	0.5	1	**10**	22	19	
68	Ofloxacin	0.5	1	5		19	
69							
70							
71	**Aminoglycosides**[1]	**MIC bre**					
72		**S ≤**					
73							
74	Amikacin	8					
75	Gentamicin	2					
76	Netilmicin	2					
77	Tobramycin	2					
78							
79							
80	**Glycopeptides**	**MIC breakpoint**		**Disk**	**Zone diameter**		**Notes**
81		**S ≤**	**R>**	**content**	**S ≥**	**R<**	Numbers for comments on MIC breakpoints





EUCAST encourages countries to form a National AST Committee (NAC).

NAC

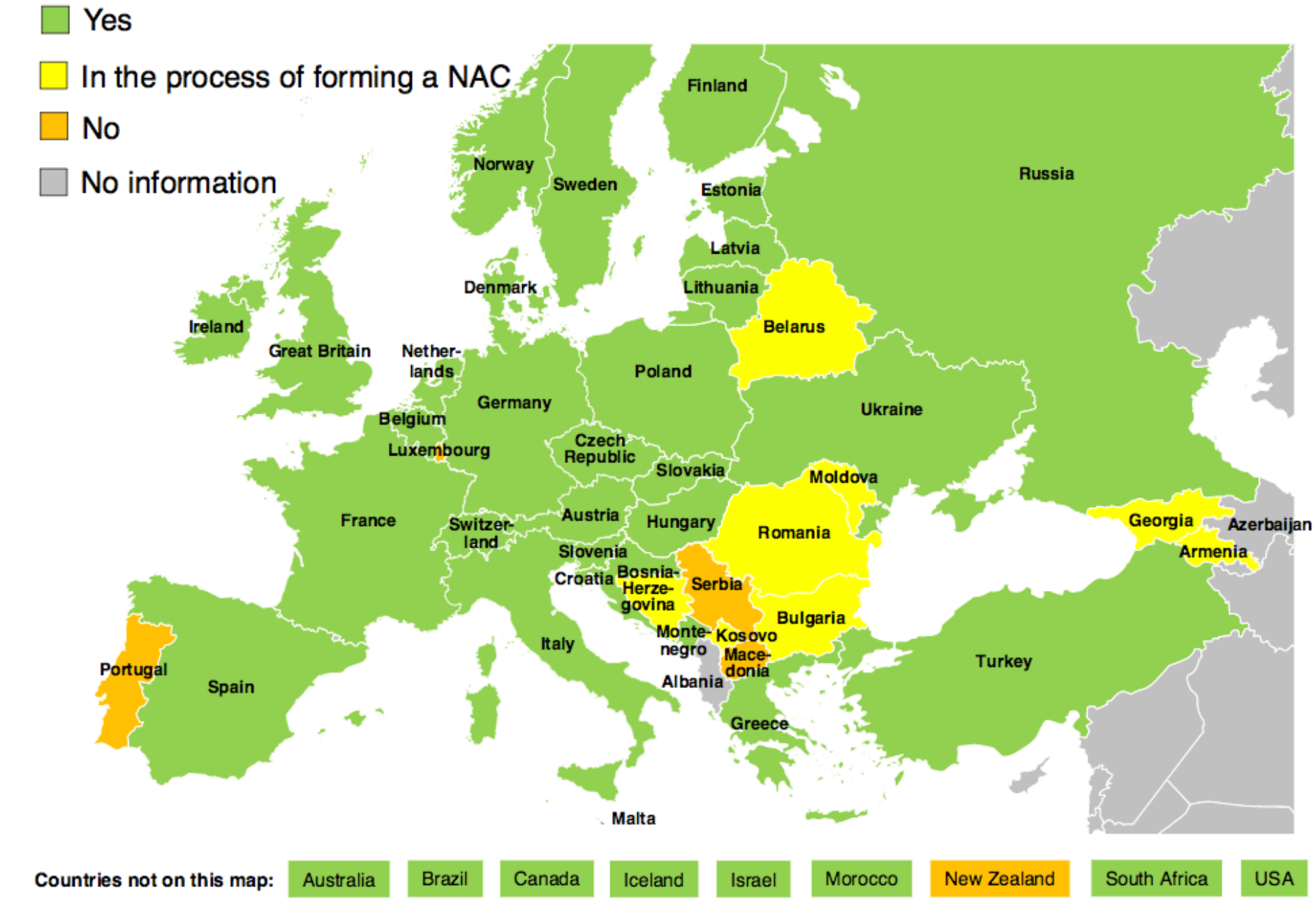
A document describing a prototype NAC is available on website.

NAC

- **Antimicrobial susceptibility testing**
  - **National strategy**
  - **Implementation** of breakpoints and methods
  - **Education** (national workshops, websites)
  - **Liaison** and **consultation** with EUCAST
  - **Translation** of documents

  - Not to deal with
    - Antimicrobial Policies
    - Antimicrobial Resistance Surveillance
    - Antimicrobial Consumption and Stewardship

National AST Committees (NACs), April 2016



EUCAST
EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING
European Society of Clinical Microbiology and Infectious Diseases

The European Committee on Antimicrobial Susceptibility Testing – EUCAST

EUCAST documents translated to other languages

- Documents in Czech
- Documents in German
- Documents in Italian
- Documents in Scandinavian languages
- Documents in Spanish
- Documents in Turkish

Documents in French (see below)

Translations in French (v 3.0):

- Implementation guideline
- Media preparation

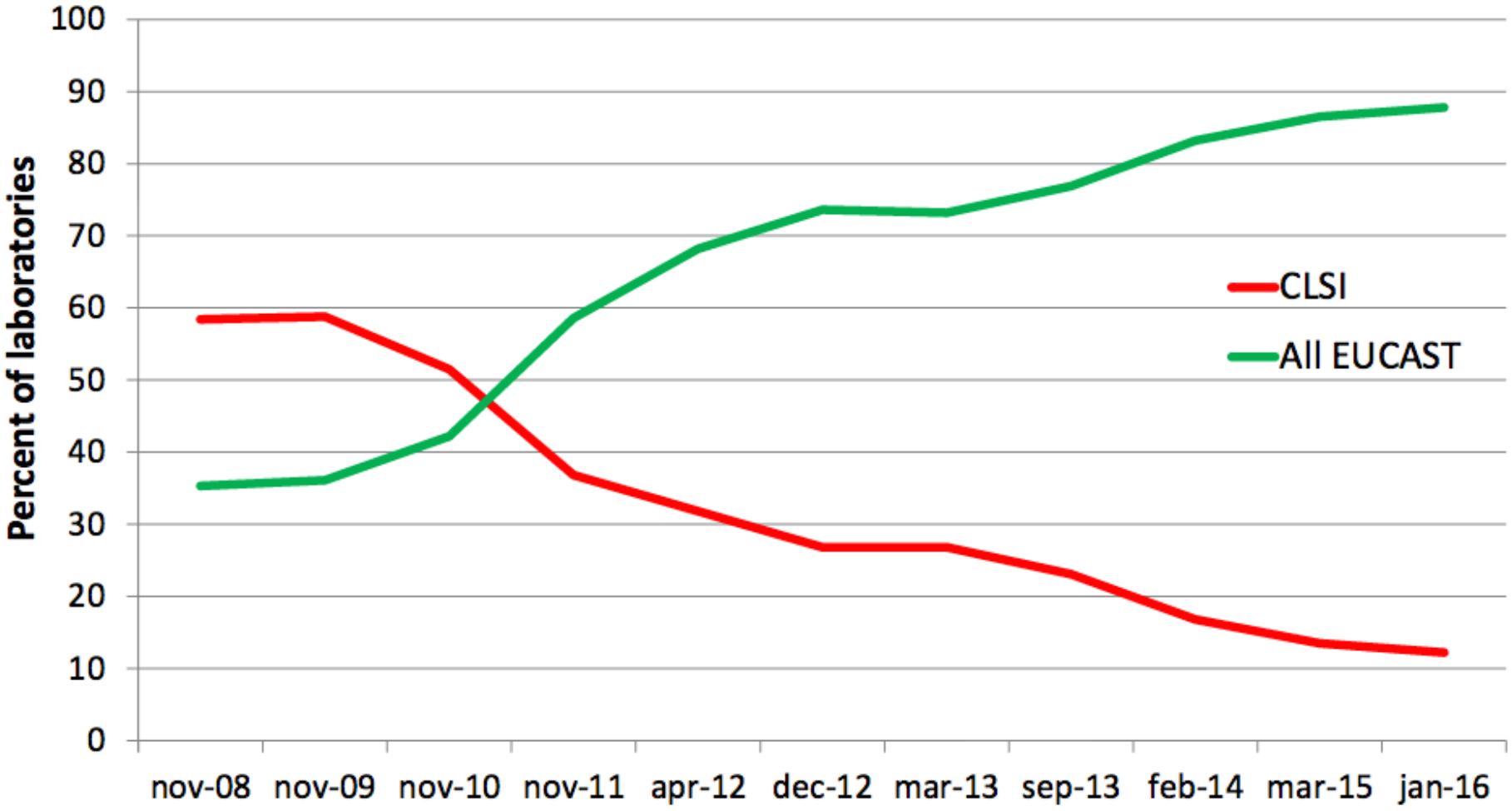
- EUCAST Disk Diffusion - Manual
- EUCAST Disk Diffusion - Slide Show
- EUCAST Disk Diffusion - Reading Guide

Implementation of EUCAST Guidelines Milestones

- 2008 when all existing antimicrobials had EUCAST breakpoints
- 2010 with the decision to develop a EUCAST disk diffusion test
- 2014 when the CA-SFM abandoned the french disk diffusion test
- 2014 when many countries outside Europe decided to turn to EUCAST and leave CLSI.
- 2016 with the publication of the uneven quality of disks from 9 manufacturers.
- 2016 when the BSAC abandoned to UK disk diffuison test.

AST guidelines used in UK NEQAS External Quality Assurance

(630-750 participants per year from a total of 40 countries)

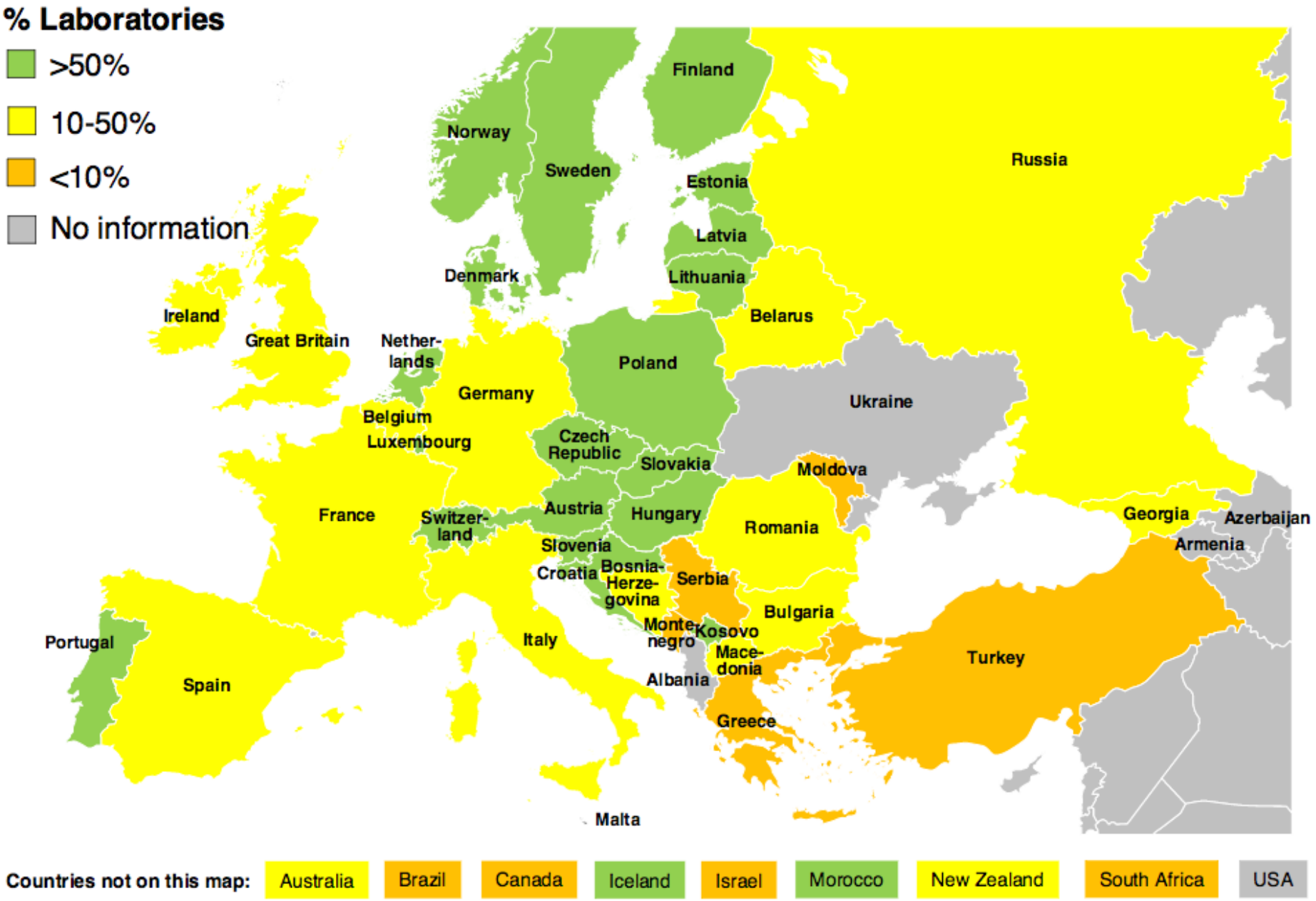


UK NEQAS

Implementation of EUCAST breakpoints, April 2016

Countries not on this map: Australia | Brazil | Canada | Iceland | Israel | Morocco | New Zealand | South Africa | USA

Adoption of the EUCAST disk diffusion method, April 2016



EUCAST –in the pipeline for 2016/17

- Colistin breakpoints and methods following the final report of the joint CLSI/EUCAST subcommittee on Colistin breakpoints
- Review and possible revision of fluoroquinolone, tigecycline and carbapenem breakpoints.
- Disk diffusion of fosfomycin, temocillin and nitroxoline
- Breakpoints and disk diffusion for *Kingella kingae, Aerococcus spp, Aeromonas* and *Plesiomonas spp*.
- Breakpoints for several new agents (betalactaminhibitor agents)
- Revised definition of Intermediate susceptibility category.
- Guidelines for companies submitting anti-mycobacterial agents
- Publication in CMI of the subcommittee on WGS (NGS) report
- Breakpoint table for rapid (4 - 8h) disk diffusion AST.
- Educational videos on AST with subtitles for EUCAST and WHO

Will we have internationally agreed breakpoints?

International standardisation?

1. Clinical breakpoints
2. MIC distributions and ECOFFs
3. Methodology

Needs for Breakpoint Harmonization

(Summary of agreement between **CLSI** and **EUCAST** breakpoint criteria for 2013)

Organisms	No. assessed		Same breakpoints		Overall agreement (%)
	Com-pounds	Criteria	Susceptible	Resistant	
Enterobacteriaceae	30	60	10	4	23.3%
P. aeruginosa	17	34	9	3	35.3%
Acinetobacter spp.	10	20	5	3	40.0%
Staphylococci	25	50	11	5	32.0%
Enterococci	5	10	2	2	40.0%
S. pneumoniae	27	60	11	11	36.7%
All results	-	234	48	28	**32.5%**

70 % disagreement between EUCAST and CLSI

Ron Jones, IDSA 2013

Breakpoint Harmonization in USA

(Summary of agreement between CLSI and USA-FDA PI criteria for 2013)

Fluoroquinolone[a]

- 82 breakpoints across 13 organism groups and six drugs
- Agreement
  - 33.3% (moxifloxacin) to 100.0% (NA) by drug
  - 54.3% for Gram-positive cocci
  - 61.7% for Gram-negative pathogens
  - **40 % disagreement between CLSI and FDA**

a. Most commonly used agents (ciprofloxacin, levofloxacin, moxifloxacin, norfloxacin, ofloxacin and nalidixic acid [NA]).

1. Clinical breakpoints

- international standardisation

- If as a concerted action – who takes the initiative?
  - WHO, UN, ISO?
  - EUCAST or CLSI?
  - Financing? Business model?

- If by evolution and "survival of the fittest"
  - is it then EUCAST or CLSI when judged on…
    - Science/credibility?
    - Decision model?
    - Influence/transparence?
    - Availability to the international community?

Conclusion

We may well be on our way to international standardisation….

Thank you

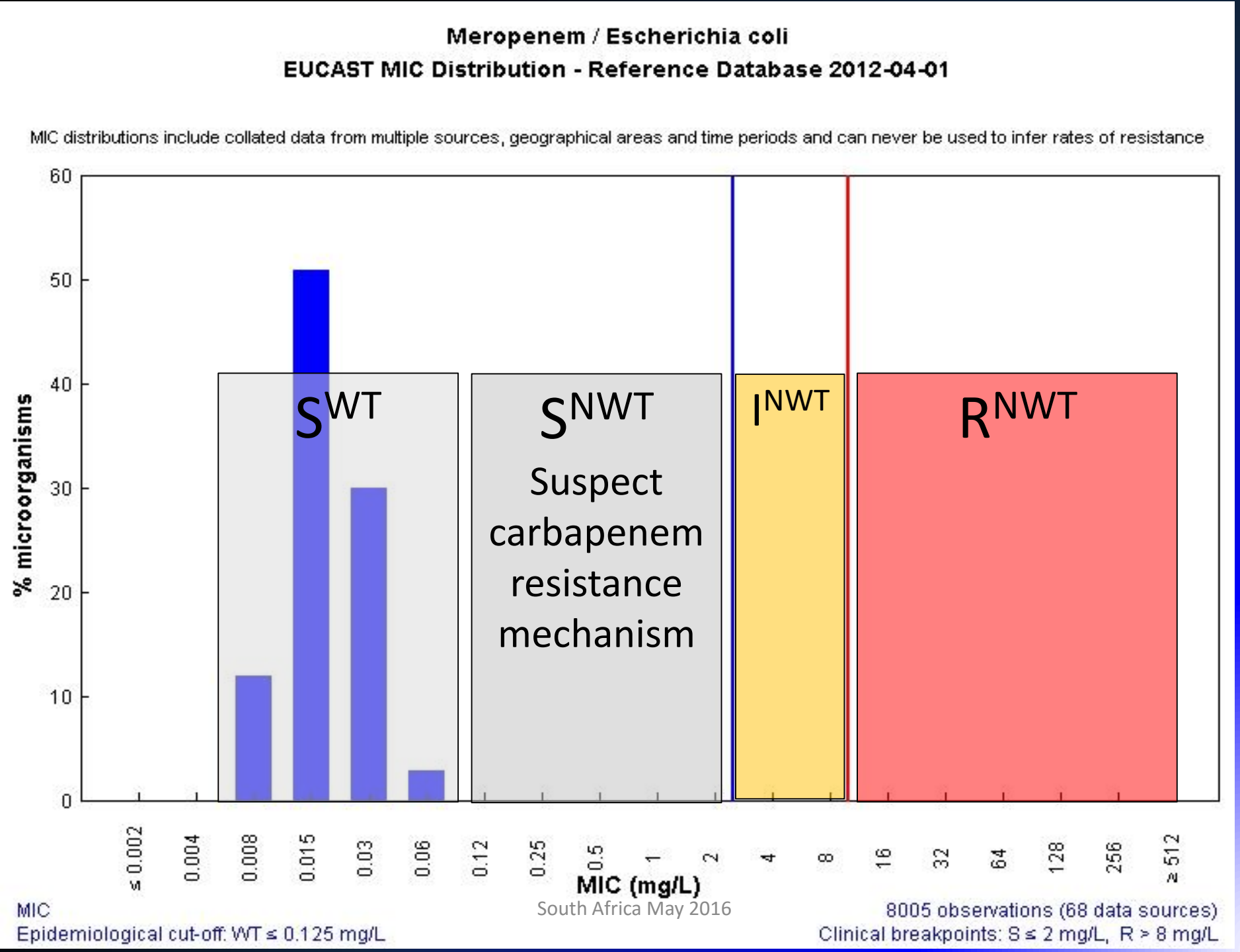

South Africa May 2016

Acknowledgements

All those who said it could not be done!

And all those who believed it could!

Thank you!


Meropenem / Escherichia coli
EUCAST MIC Distribution - Reference Database 2012-04-01
MIC distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance
% microorganisms
60
50
40
30
20
10
0
S WT
S NWT
Suspect carbapenem resistance mechanism
I NWT
R NWT
≤ 0.002
0.004
0.008
0.015
0.03
0.06
0.12
0.25
0.5
1
2
4
8
16
32
64
128
256
≥ 512
MIC (mg/L)
MIC
Epidemiological cut-off: WT ≤ 0.125 mg/L
8005 observations (68 data sources)
Clinical breakpoints: S ≤ 2 mg/L, R > 8 mg/L

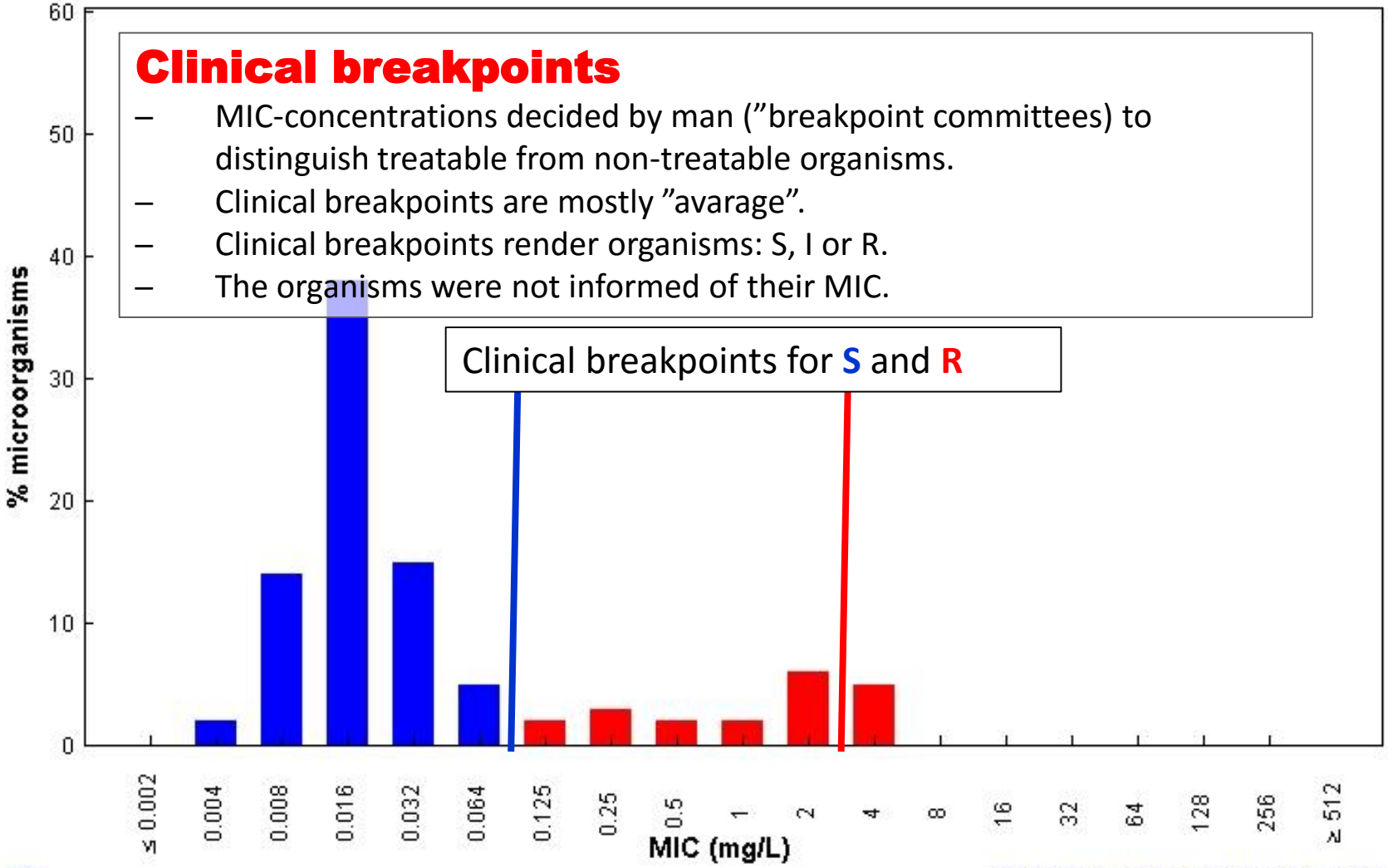
Benzylpenicillin / Streptococcus pneumoniae

EUCAST MIC Distribution - Reference Database

MIC distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance

Clinical breakpoints

- MIC-concentrations decided by man (”breakpoint committees) to distinguish treatable from non-treatable organisms.
- Clinical breakpoints are mostly ”avarage”.
- Clinical breakpoints render organisms: S, I or R.
- The organisms were not informed of their MIC.



MIC

Epidemiological cut-off: WT ≤ 0.064 mg/L

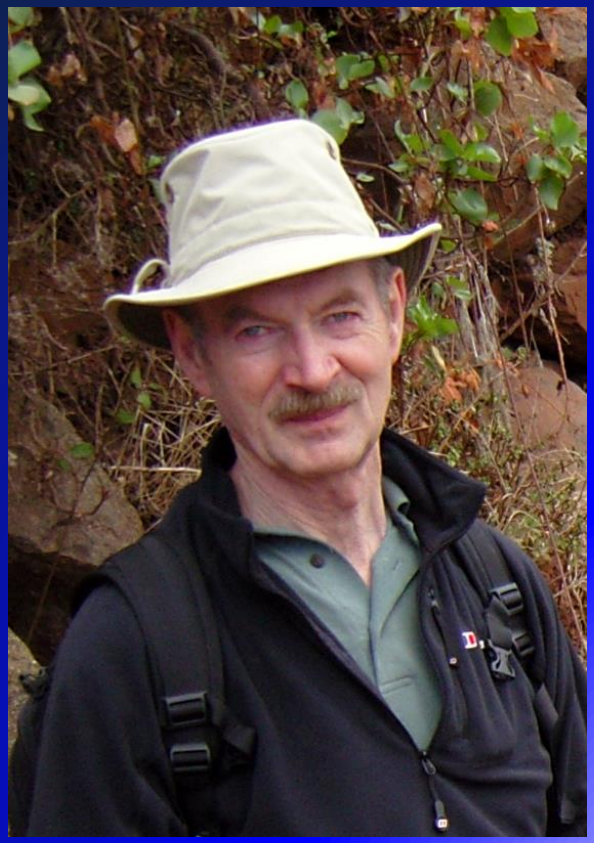
37642 observations (32 data sources)

Clinical breakpoints: S ≤ 0.064 mg/L, R > 2 mg/L

Whole genome sequensing for AST – pros and cons

- Yes/No-answer (similar to ECOFFs – 99.7%)
- Breakpoints not necessary
- Direct testing in clinical materials
- Quantitation not possible, but theoretically certain genes can be labeled “of less importance”.
- Detection only of known genes.
- Silent genes and genes coding for inducible mechanisms not distinguished.

Ian Phillips
1st EUCAST Chairman



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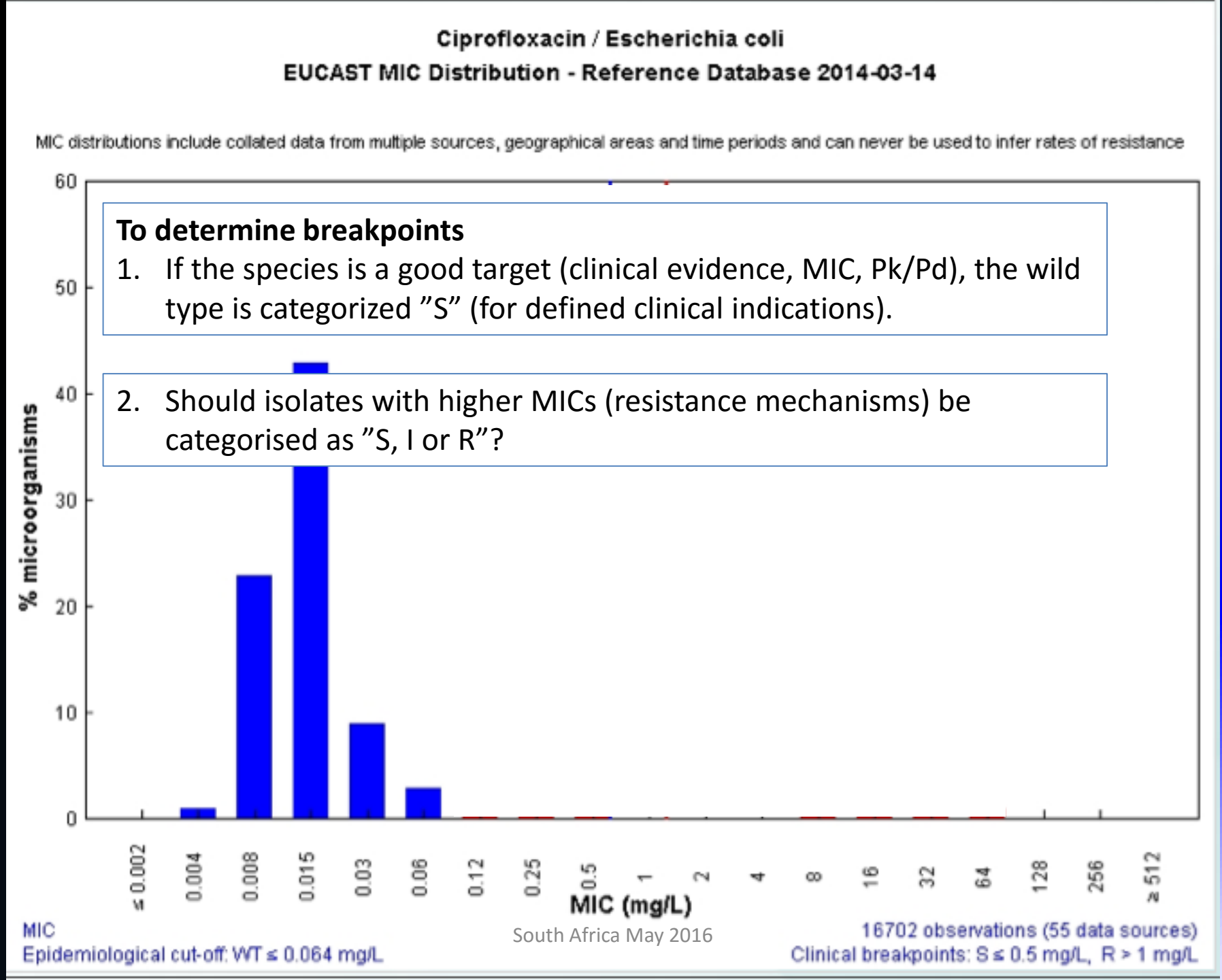
South Africa May 2016

South Africa May 2016

NCCLS meeting
Somewhere along the line I was promoted to first gunner!


Ciprofloxacin / Escherichia coli
EUCAST MIC Distribution - Reference Database 2014-03-14
MIC distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance
To determine breakpoints
1. If the species is a good target (clinical evidence, MIC, Pk/Pd), the wild type is categorized ”S” (for defined clinical indications).
2. Should isolates with higher MICs (resistance mechanisms) be categorised as ”S, I or R”?
% microorganisms
60
50
40
30
20
10
0
≤ 0.002
0.004
0.008
0.015
0.03
0.06
0.12
0.25
0.5
1
2
4
8
16
32
64
128
256
≥ 512
MIC (mg/L)
MIC
Epidemiological cut-off: WT ≤ 0.064 mg/L
16702 observations (55 data sources)
Clinical breakpoints: S ≤ 0.5 mg/L, R > 1 mg/L

Useful Abbreviations, Acronyms

- **ECDC** – the European Centre for Disease prevention and Control (European **CDC**)
- **EMA** – the European Medicines Agency (European FDA)
- **ESCMID** – the European Society of Clinical Microbiology and Infectious Diseases (European ASM+IDSA)

- **EUCAST** – the European Committee on Antimicrobial Susceptibility Testing (BSAC, CA-SFM, CRG, NWGA, SRGA
- **NAC** – National AST Committee
- **ECOFF** – the Epidemiological Cut Off value

MIC

Measured as growth inhibition (naked eye) by a concentration of the agent in a standardised broth micro dilution system based on two fold dilutions where the concentration 1 mg/L is mandatory.

…or a surrogate measure such as

- an inhibition zone diameter
- a gradient test inhibition elliptical inhibition zone

EUCAST and CLSI are different

EUCAST

- Profession together with regulatory authorities
- Funded by ESCMID, ECDC and national breakpoint committees.
- Industry consultative role.
- Decision by consensus.
- Five meetings per year.
- EUCAST=EMEA brpt committee.
- Clinical breakpoints and ECOFFs
- Rationale for decisions published
- Documents in public domain and free of charge

CLSI

- Industry, the profession, advisory regulators.
- Funded by industry and sales of output.
- Industry part of decision process
- Decision by vote.
- Two meetings per year.
- CLSI technical standing with FDA.
- Clinical breakpoints
- Rationale for decisions not published.
- Documents for sale

The basic difference between European and US philosophy in breakpoint setting:

- Europe – demonstrate that it works and we may consider raising the breakpoint!

- USA – demonstrate that it fails and we may consider lowering the breakpoint!

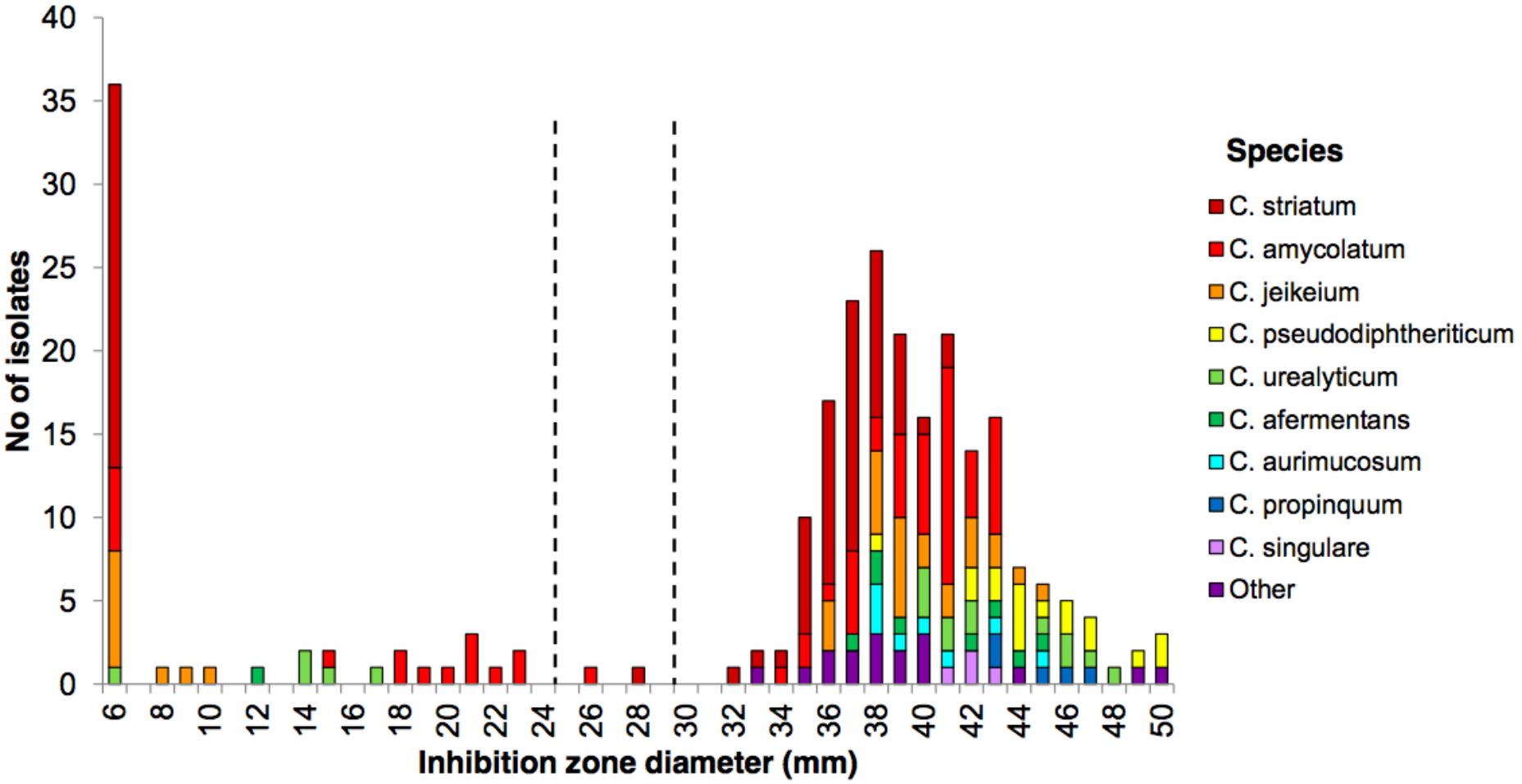
Is MIC (mg/L) a more robust measurement than the inhibition zone diameter?

- MIC determination (whether right or wrong) provides a definitive answer, which is attractive to many – only rarely are you in a position to question an MIC (ability to perform another method, cost).
- MIC values are discontinuous variables (as opposed to inhibition zone diameters)
- Because of this, reproducibility of MIC testing is mostly considered to be +/-1 dilution step (which for some is a little pessimistic).
- This corresponds to +/-3 mm in the disk diffusion test.

Reproducibility of AST with phenotypical test systems

- Variations in materials (MH, disks, antibiotics)
- Variations in the test system (time, temperature, reading end points)
- Breakpoints vs. MIC (or zone diameter) distribution

Rifampicin 5 μg vs. species
Corynebacterium spp., 254 clinical isolates



Breakpoints
Zone diameter S≥30, R<25 mm

USA, FDA and NCCLS

- When commenting on the FDA's decision to go for the Kirby–Bauer disc method, Maxwell Finland, a specialist in internal medicine and leading therapeutic reformer, called it "an 'arbitrary decision reached by FDA after consultation with a small number of consultants. Some of the details of the decision are difficult to accept"

History of AST Methods (The Beginning)

- Fleming's "ditch-plate" test (1929)[a]
- From the ditch to the well to the disk (1960)[b]; and dilution (MIC) tests in agar and tubes
- Road to standardization
  - WHO working groups formed since 1960[c]
  - Publication of the International Collaborative Study (ICS)[d]
  - USA-FDA modified ICS and Bauer-Kirby (1966) disk methods[e], a method to become NCCLS (CLSI) M2-A, via A.L. Barry et al.

a. Fleming, A. 1929. Br. J. Exp. Pathol. 10:226-236.
b. Ericsson, H. 1960. J. Clin. & Lab. Invest. 12:1-15.
c. World Health Organization. 1961. World Health Organization Technical Reports Series No. 210, pp. 1-24.
d. Ericsson, H.M. and J.C. Sherris. 1971. Acta Pathol. Microbiol. Scand. Section B. Suppl. 217.
e. Federal Register. 1973. Fed. Regist. 38:2576.

Enterobacteriaceae 1975 – 2001

Committee	Amoxicillin	Cefotaxime	Piperacillin-tazob.
BSAC (UK)	8 / 16	2 / 2	16 / 16
CA-SFM (F)	4 / 16	4 / 32	8 / 64
CRG (NL)	2 / 16	4 / 8	0.25 / 4
DIN (D)	2 / 8	2 / 8	0.12 / 1
NCCLS (USA)	8 / 16	8 / 32	16 / 64
NWGA (N)	0.5 / 8	1 / 2	8 / 16
SRGA (S)	1 / 8	0.5 / 1	16 / 16
EUCAST (2008)	8 / 8	1 / 2	8 / 16

All of us managed to come up with different breakpoints.

3. Breakpoints by pharmaceutical companies

- Companies suggest breakpoints (to medicines agencies and/or breakpoint committees)
- Companies can require medicines agencies to change existing breakpoints – but there is no real review process to right unfortunate decisions (and often agents are generic and the sponsor is lost)

4. Breakpoints by AST companies

- Most automated systems are Susceptibility Testing Machines – where output is S, I and R – not MIC.
- Changing breakpoints in automated systems is a tedious procedure – and sometimes take years. The unfortunate user is left to his own devices for long periods of time.

ECOFF vs. Clinical breakpoint

- If the WT has been deemed an appropriate target for the agent, the ECOFF is the lowest possible value for a clinical S-breakpoint.
- If there are no resistant isolates or no clinical data to support a higher clinical breakpoint than the ECOFF, the ECOFF will be used in lieu of a clinical breakpoint.
- The ECOFF in itself does not categorise wild type distributions as susceptible.

- Formed in 1997 by ESCMID
  - Chairpersons
    - Ian Phillips, UK, 1997 – 2001
    - Gunnar Kahlmeter, Sweden, 2001 – 2012
    - Rafael Canton, Spain, 2012 –
  - Scientific secretary
    - Derek Brown, UK, 1997 – …..
- Restructured in 2001-02
- General Committee with representatives from all European countries and Australia and the USA.
- A Steering Committee with chair, scientific secretary and clinical data coordinator appointed by ESCMID and with representatives from national breakpoint committees and the General committee.

The ICS, commissioned by WHO, and with representatives from many European countries (1962 – 1971):

Its objective is described in the Expert Committee on Antibiotics' report - the universal adaption of reliable methods, standardized as far as possible, would have the following three advantages:

- It would afford the best possible guidance to the clinician in the treatment of his patients.
- It would enable comparative assessments to be made of the frequency, importance and epidemiology of resistant strains of bacteria in different institutions, areas and countries.
- It would facilitate the interpretations of published findings, which often cannot be compared with those of other workers.